COMPARISON OF OSMOREGULATION IN TWO SPECIES OF THE GENUS **Fundulus**

By

DALE FREDERICK BURNSIDE

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For

MY PARENTS
who gave me life

and for

LAURA
who shares my life

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INTRODUCTION

The integrity of any organism is maintained by the functioning of homeostatic systems. Various parameters are physiologically controlled at levels differing from environmental levels. Because an organism is an open system, energy is required not only to establish these internal levels but also to maintain them. The energetic cost of regulating any parameter is a function of:

1. the difference between the maintained level and the environmental level
2. the degree to which the animal is open to the parameter
3. the efficiencies of the controlling mechanisms.

The osmoregulatory system is one by which body fluid concentration can be maintained at a relatively constant level irrespective of variations in the concentration of the external medium. General aspects of osmoregulation have been reviewed by Krogh (1939), Hober _et. al_. (1945), Baldwin (1948), and Potts and Parry (1964). Osmoregulation in teleost fishes has been reviewed by Smith (1932), Black (1951, 1957), Gordon (1964), and Parry (1966).

To study the problem of teleost osmoregulation, I have chosen two congeneric Cyprinodont species which inhabit areas of different salinity conditions. The euryhaline _Fundulus similis_ (Baird and Girard) lives in salt marshes where salinity conditions are highly variable.

The comparatively stenohaline Fundulus chrysotus (Holbrook) has been found occasionally in waters of up to 24.7 ppt (Kilby, 1955), but large populations are restricted to fresh water.

The energetic cost of osmoregulation in fishes is the sum of the transport work performed by the kidneys, gills, and intestinal tract. The gills account for the majority of the energetic expenditure (Margaria, 1931; Potts, 1954). In order to estimate the energetic cost of osmoregulation, I have measured the oxygen consumption of intact animals and excised gills as a function of the concentration difference between the animal and the environment.

The degree to which the osmoregulatory system of an animal is open to the environment is termed permeability. Because the energetic efficiencies of the controlling mechanisms are not considered in this study, they have been combined with permeability to yield a compound factor which I call apparent permeability. If the efficiencies are high or the permeability is low, the apparent permeability is low. If the efficiencies are low or the permeability is high, then the apparent permeabiltiy is high. Apparent permeability varies directly with permeability and inversely with the efficiencies of the controlling mechanisms.

Parry (1958) has suggested that body size is an important factor affecting the ability to osmoregulate. This factor has been almost completely ignored in osmoregulatory studies. In the present study, body size is considered by making plots of log oxygen consumption-log body weight at different salinities. This type of analysis has been carried out by Hickman (1959) for fishes and by Gilchrist (1956), Rao (1958), and Lumbye (1958) for various invertebrates. For tissue respiration studies, the dry gill tissue weight is correlated with oxygen consumption and with body weight in order to consider the influence of body size.

Purposes

The purposes of this study are:

1. to determine the precision of osmoregulation of two closely related species of fish which are from habitats of different salinity conditions
2. to consider the energetic cost of osmoregulation for these two species by measuring the oxygen consumption of intact animals and excised gills as a function of salinity
3. to calculate apparent permeability as a function of body size and salinity
4. to compare the magnitude and direction of changes in the oxygen consumption of intact animals and excised gills as a function of body size and salinity.

MATERIALS AND METHODS

Collection, Maintenance, and Acclimation of Fish

Seines were used to collect *F. similis* from tidal creeks near Cedar Key, Florida. The salinity at times of collection varied from 8 to 34 ppt. Specimens of *F. chrysotus* were collected in the Gainesville, Florida,area from Lake McCloud and River Styx with dipnets, seines, and small dredges.

Fish were maintained in 10-gallon aquaria. Water was aerated and filtered. Temperature was regulated between 20 and 25° C. During the studies with intact animals, a 12-hour light and 12-hour dark cycle was maintained. For the blood plasma studies and tissue metabolism experiments, light was not controlled. Fish were fed commercial shrimp pellets.

F. similis was acclimated to the different salinities in a stepwise fashion. Usually the salinity at collection was near that of full sea water. Therefore, the fish were first kept in full sea water from Marineland® of Florida (35.5 ppt). Two days later, 3/4 of the fish were transferred to a dilution of 2/3 sea water to 1/3 fresh spring water. In another two days, 2/3 of these fish were transferred from 2/3 sea water into a 1/3 sea water mixture. Because *F. similis* could not survive 100% spring water, 1/2 of the fish in 1/3 sea water were transferred into a mixture equaling ca. 1 ppt salinity. After all transfers had been completed, a period of two weeks was allowed for full acclimation. Parry (1966) has suggested that only a few hours are required for fish to become acclimated to salinity.

F. chrysotus was also acclimated in a stepwise fashion. The process began in fresh water and proceeded into 1/3, 2/3, and full sea water. A period of two days was allowed between transfers and a period of two weeks was allowed for full acclimation.

Plasma Samples

Plasma samples were obtained using heparinized capillary tubes. Fish were removed from the aquaria and excess moisture in the gill region was absorbed with tissue paper. A heparinized capillary tube (1.4-1.6 mm in diameter) was placed adjacent to the isthmus and gently twisted and pushed into the heart. When the capillary tube met reduced resistance and the heart had been pierced, the tube was slightly retracted. Whole blood entered the tube.

Samples were centrifuged at 10,000 rev/min for 15 minutes to separate the plasma from the blood cells. For large samples (all *F. similis* and some *F. chrysotus*), the plasma was separated from the cells by breaking the tube at the plasma-cell interface. Then the plasma was transferred to a new capillary tube. Such a separation was too difficult for small samples and the plasma was frozen in a vertical position with the column of cells under it.

Determination of Blood Plasma Concentration

The determination of blood plasma concentration was modeled after Gross (1954), Hoar (1960), and Pavlovskii (1964). This method is performed by comparing the time of melting of frozen solutions of unknown osmolar concentration with solutions of known osmolar concentration when allowed to warm very slowly and linearly in a cold brine (saturated NaCl) bath. Because only 0.001-0.01 ml of sample is needed to fill a small portion of a capillary tube, this method is desirable for small quantities.

A 1-gallon battery jar was suspended in a 2.5-gallon bucket which had a 12.5 cm diameter hole in the bottom. Styrofoam® insulation was placed between the bucket and battery jar. The bucket was elevated by three legs. Reflected light was passed through the battery jar containing cooled brine. One polaroid plate, 0.030 inches thick and 30% light transmission, was placed immediately below the battery jar and another was placed above the battery jar in the "crossed" position. This allowed 1% light transmission and caused crystals to appear to glow. Gentle circulation of the brine was provided by an air-driven stirrer. For suspending samples in the battery jar, a capillary tube holder was made from a plastic soap dish.

Standard solutions were mixed to known concentrations as follows according to Harned and Owen (1958, page 492):

Molarity	Osmotic Coefficient	Osmolarity
0.1 NaCl	1.86	.186
0.2 NaCl	1.84	.368
0.3 NaCl	1.83	.549
0.5 NaCl	1.81	.905

An experimental run consisted of these four standards plus two to four unknowns. All volumes were approximately equal. The standards and unknowns were placed in the holder and frozen with dry ice. The brine was cooled to between -8 and -10° C with dry ice. The capillary tube holder was suspended in the cooled bath and the polaroid plates were positioned. The bath was allowed to warm at the rate of ca. 1° C every half hour during the critical temperature range from -2 to 0° C. The thawing of ice crystals was observed and time was recorded as the last crystal melted.

Then the concentrations of the unknowns could be determined from a graph of osmolar concentration and time of thawing of standards. The accuracy of this method is ± 5-10 mosm/liter.

Activity

The system for measuring the effects of experimental handling on the activity of fish consisted of a 6.3 volt light source with an infrared filter and a Hunter® photorelay connected to an Esterline Angus® event recorder.

The experimental fish was placed in a flask (either 250 or 500 ml, depending upon the size of the fish) between the infrared light source and the photorelay. Whenever the fish broke the beam, the event was recorded. During an experiment, water was kept aerated with an airstone and temperature was maintained constant at 20° C. All experiments were continued for 24 hours in the dark.

Five individuals of each species were used. The number of events on the recorder paper was grouped into 1-hour intervals. Data from each of five fish of the same species were added together for each hour interval. The total number of times that the beam was broken per hour was graphed against time. The period of minimal activity was thus determined.

Intact Animal Oxygen Consumption

Apparatus

The system for measuring the oxygen consumption of intact animals was modified from that of Fry and Hart (1947).

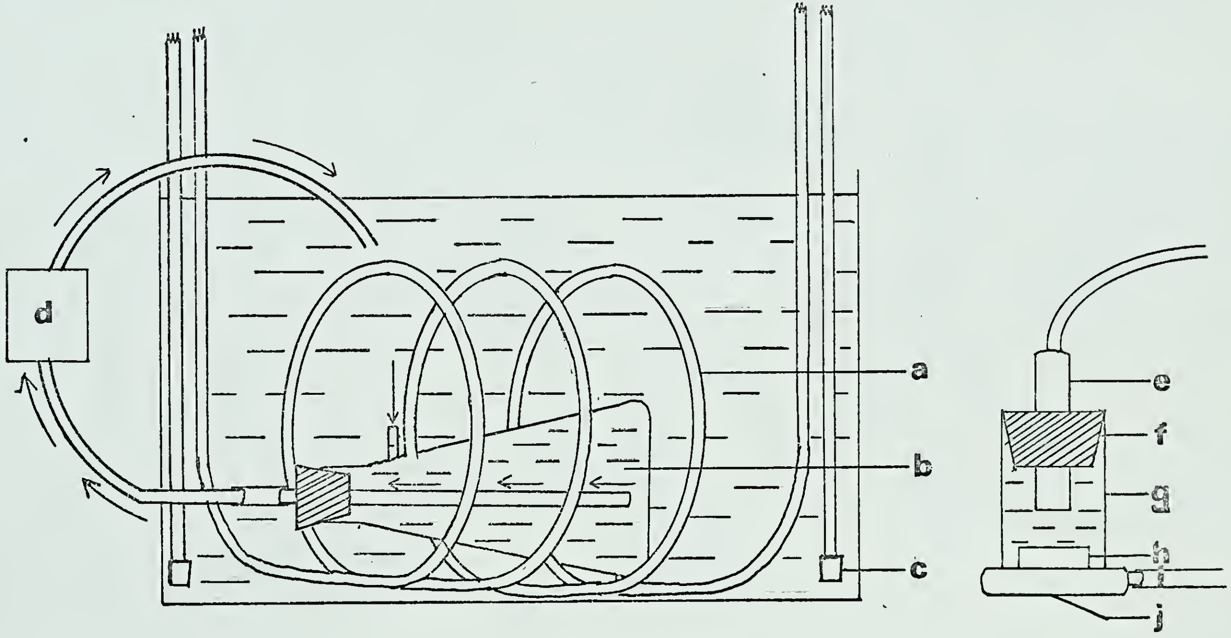
The respiration chamber consisted of a one-arm suction filtration flask fitted with a one-hole rubber stopper through which a glass tube was passed. The end of the glass tube inside the flask was positioned as far away from the arm as possible (see Figure 1). Two sizes of flask were used (250 and 500 ml), depending upon the size of the fish.

A 19-liter aquarium served as both the temperature bath and the resevoir for the circulating medium. One end of the aquarium was removed and replaced by plexiglass through which a hole was drilled. A glass tube, 5 cm long, was passed through this hole around which aquarium cement was applied to prevent leakage. Both ends of the glass tube were connected to tygon tubing. The longer tube on the outside of the aquarium passed through a circulation pump and back into the aquarium. The shorter tube on the inside was connected to the glass tube of the respiration flask. When the circulation pump was turned on, water left the flask via the glass tubing and was replaced by water entering through the arm of the flask. See Figure 1 for flow directions.

Water circulated through the cooling coil from a constant temperature bath held at 20° C. An air pump vented through airstones maintained a high oxygen concentration in the aquarium water.

To provide insulation and darkness, the aquarium was placed in a large Styrofoam® cooler which was painted flat black on the inside.

Figure 1. System for measuring the oxygen consumption of intact animals. (a) cooling coil; (b) filtration flask; (c) airstone; (d) circulation pump; (e) oxygen probe; (f) rubber stopper; (g) 100-ml sample bottle; (h) magnet; (i) air hose; (j) magnetic stirrer.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Determination of Dissolved Oxygen

The Precision® galvanic cell oxygen analyzer reads linearly in milliamperes of electricity flowing between cathode and anode. It measures oxygen concentration to within 0.1 ppm at constant temperature. The analyzer's flow requirement of 1.0 ft/sec was met with an electromagnetic stirrer (see Figure 1).

Approximately 200 ml of water at the same temperature and salinity as the water in the 19-liter aquarium was vigorously aerated overnight. A Winkler determination for dissolved oxygen indicated that this water was consistently 99-100% saturated. This water was used as a standard of reference for the oxygen analyzer readings.

Readings in milliamperes were taken for 100 ml of the standard water and for 100 ml of an unknown sample. The following calculation yielded dissolved oxygen in the unknown sample:

$$\frac{\text{milliamperes-sample}}{\text{milliamperes-standard}} \times \begin{array}{l}\text{amount dissolved at saturation}\\ \text{for given temperature and salinity}\end{array}$$

Saturation values were read from a nomogram which was supplied with the oxygen analyzer.

Experimental Procedure and Analysis

The experimental animals were fasted for 24 hours prior to their use. Each fish was placed in a full respirometer flask which was stoppered and connected to the tygon tubing inside the aquarium. The circulation pump was turned on and adjusted to give a flow rate of 100-150 ml/min. This rate had no visible effects on the fish and was fast enough so that the water in the flask would stay as well aerated as the water in the aquarium. The fish was allowed to remain in this condition until the period of minimal activity had been reached.

At the beginning of an experiment, a 100 ml sample of water was removed through the outside tygon tubing and oxygen concentration was determined. The system was then stopped from flowing for 30 minutes and a second sample was then taken from inside the flask. The dead space (tygon and glass tubing) was cleared and a 100 ml water sample was collected through the outside tube. The oxygen concentration was determined and subtracted from the first determination. The system was then allowed to flow for 30 minutes and the entire procedure was repeated from the beginning. Five such determinations were made during a 4.5-hour period. Fish were superficially dried with paper towels and weighed after experiments on a Torsion® balance to a precision of 0.01 g.

Experiments with blue dye demonstrated that no mixing of water entering and leaving the flask occurred while drawing samples.

The average of five readings was considered to be an estimate of rountine oxygen consumption as defined by Beamish and Mookherjii (1964). The log of oxygen consumption (ml/hr) was correlated with the log of body weight (g) for each species at four different salinities.

$$\text{Log oxygen consumption} = A \log \text{body weight} + \log B$$

where:

A = increase in log oxygen consumption relative to increase in log body weight

B = amount of oxygen consumed by a 1.0 g fish.

Special Considerations

Fry (1957) has suggested that the following variables may affect the oxygen consumption of fish:

1. experimental temperature and thermal history
2. oxygen and carbon dioxide tensions

3. seasonal influences
4. diurnal variations
5. activity
6. nutritional state
7. sex
8. body weight
9. environmental salinity.

In this study, an attempt was made to consider each of these factors and to allow only body size and environmental salinity to vary. Diurnal variations, sex, and seasonal influences are not discussed elsewhere in this paper. Experiments were performed between 0800 and 1200 (Eastern Standard Time) and between 2000 and 2400 (Eastern Standard Time). No difference was apparent in the results between these two groups. Sex of individuals was determined, but was found not to significantly affect oxygen consumption. The duration of these studies did not permit the observation of possible seasonal influences on oxygen consumption.

Oxygen Consumption of Excised Gills

Experimental Setup

The oxygen consumption of excised gills was determined using a Gilson differential respirometer. This is a closed system in which the reaction vessels are separated from a compensation chamber by a manometer (Umbreit *et al.*, 1956). Fifteen ml capacity flasks were used. The center well of the flask contained a piece of folded filter paper which had been saturated with 10% KOH. The main compartment of the flask contained the gills and 2.0 ml of water of the desired salinity.

Experimental Procedure

The gill apparatus was excised as a unit. A single cut was made at the caudal end of the isthmus. The isthmus was pulled cephalad exposing the gill apparatus. After the gill apparatus had been teased loose using a scapel and dissecting needles, it was separated from the fish with a forceps and divided into right and left halves. This division allowed the tissue to lie flat in the manometer vessels and be completely covered by the medium.

Oxygen consumption was corrected to standard conditions. Measurements were made at 20° C and barometric pressure was recorded at the beginning and end of each experiment.

A period of thirty minutes was allowed for equilibration and then readings were taken at 15-minute intervals for a period of 2.0 hours. At the end of each experiment, the gills were removed from the manometer vessels and the surplus liquid was absorbed from the tissue with paper towels. The gills were placed in small unstoppered vials and dried for one week at 110° C in a drying oven. At which time, the dry tissue weight was determined with a Mettler® analytical balance to a precision of 0.1 mg.

Analysis

For each species in each medium, a plot of log dry gill weight (mg) against log oxygen consumption (μl/hr) was made.

Log oxygen consumption = A log dry gill weight + log B.

The wet body weight of each fish was also recorded and log dry gill weight (mg) was plotted as a function of wet body weight (g).

Log dry gill weight = A log wet body weight + log B.

Special Considerations

Krebs (1950) has discussed two important aspects of tissue metabolism:

1. the choice of medium
2. treatment of tissues.

In the present study, the medium was an artificially prepared sea water (Svedrup *et al.*, 1942) and dilutions were made with distilled water to produce salinities which would be consistent with those of the blood plasma studies. The gills were excised as an intact unit and tissue damage was minimal.

Gill Surface Area

The method of determining the surface area of the gills was according to Gray (1954) and Hughes (1966). Intact fish were weighed and their gills removed. The number of filaments on each gill arch was counted for the gill apparatus on one side of the fish. The average length of these filaments was determined by measuring every fifth filament on each gill arch. The average number of lamellae per mm was determined by making five counts on the filaments of each gill arch. The average area of the lamellae was determined by sampling ten lamellae per fish. These were drawn by means of a Zeichentubus® and a planimeter was used to determine surface area. The lamellae are triangular in cross section. Because these drawings are two dimensional, their areas were multiplied by a factor of three to get total surface area. It is believed that this is an underestimate of the true surface area because the triangular cross sections have curved sides. The formula for gill surface area is:

$$SA = 8 \times \overline{\#F} \times \overline{L} \times \overline{l} \times \overline{sa}$$

where:

SA = total gill surface area in mm^2

8 = total number of gill arches

$\overline{\#F}$ = avaerage number of filaments per gill arch

$\overline{L}$ = average length of filaments in mm

$\overline{l}$ = average number of lamellae per mm

$\overline{sa}$ = average surface area of lamellae in mm^2.

For each species, log surface area (mm^2) was correlated with log wet body weight (g).

Log surface area = A log wet weight + log B.

Fish and gills were stored in 10% formalin during these measurements and correction factors for shrinkage and weight change were applied according to Parker (1963).

Permeability

Gill permeabilities were inferred from other data by using the equation of Potts (1954):

$$W = KA\,(B-M)\,R\,T\,\ln B/M$$

where:

W = work in cal/hr

K = permeability in moles/mm^2-molar difference-hr

A = surface area in mm^2

B = blood concentration in moles/liter

M = medium concentration in moles/liter

R = 1.987 cal/°C-mole

T = absolute temperature in °K.

This equation may be rewritten:

$$K = \frac{W}{A\ (B-M)\ R\ T\ \ln B/M}.$$

Work was derived from gill oxygen consumption readings by multiplying data by 4.8 cal/ml O_2. It was decided better not to subtract a small amount of energy used for maintenance of the gill cells, because Harvey et al. (1967) have shown that this portion of the metabolism is also instrumental in ion transport. Surface area of the gills was known from the relationships in Table 18. Molar concentrations of the medium and blood were calculated from osmolar concentration data.

Assumptions upon which these calculations are based are considered in the discussion.

Statistical Methods

Regression lines were calculated according to the least squares method for curvilinear regression (Miller and Freund, 1965).

Regression coefficients are given with one standard error of the estimate and are compared by the method given in Bailey (1959).

Intercepts are given with one standard deviation according to Snedecor (1965). Both the variation around the sample mean and the variation of the regression coefficient are taken into account. Comparison of intercepts involved the use of "pooled" variances from the regression and the test statistic for intercepts according to Miller and Freund (1965).

Sample standard deviations from regressions were calculated for each regression line according to Snedecor (1965).

Means of the blood plasma samples were compared using a standard "t" test statistic for hypotheses concerning two means after Miller and Freund (1965).

RESULTS

Activity

Both species are most active immediately after being placed in the respiration flasks. Activity then decreases to a minimal level and eventually increases until the end of the experiment (see Figures 2 and 3). Between 11 and 15 hours after the beginning of the experiments, *F. similis* exhibits minimal activity which is approximately 40% of the original level of activity. The period of minimal activity for *F. chrysotus* occurs between 7 and 18 hours after being placed in respiration flasks, and is less than 1% of the original activity. Measurements of rountine oxygen consumption were made during these periods of minimal activity.

Concentration of Blood Plasma

As the concentration of the medium is raised from 25 mosm/liter to 1,000 mosm/liter, the concentration of the blood plasma of *F. similis* increases from 339 ± 15.9 mosm/liter to 397 ± 7.1 mosm/liter (Figure 4). At $[M] = 370$ mosm/liter, the mean fish plasma concentration equals that of the medium. All means are significantly different from each other except those of fish in 1/3 and 2/3 sea water.

The concentration of the blood plasma of *F. chrysotus* increases from 246 ± 22.8 mosm/liter to 459 ± 26.3 mosm/liter as the medium

Figure 2. Activity record of *F. similis* after placement in respiration chambers. Data are the totals from records of five fish. The period of minimal activity is indicated.

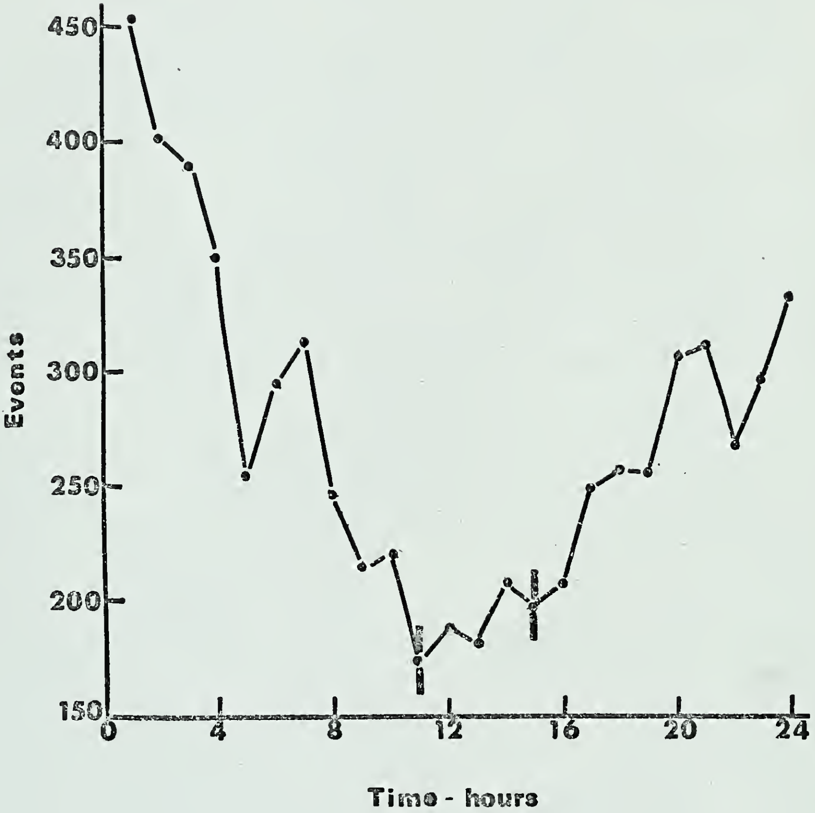

Events
450
400
350
300
250
200
150
0
4
8
12
16
20
24
Time - hours

Figure 3. Activity record of _F. chrysotus_ after placement in respiration chambers. Data are the totals from records of five fish. The period of minimal activity is indicated.

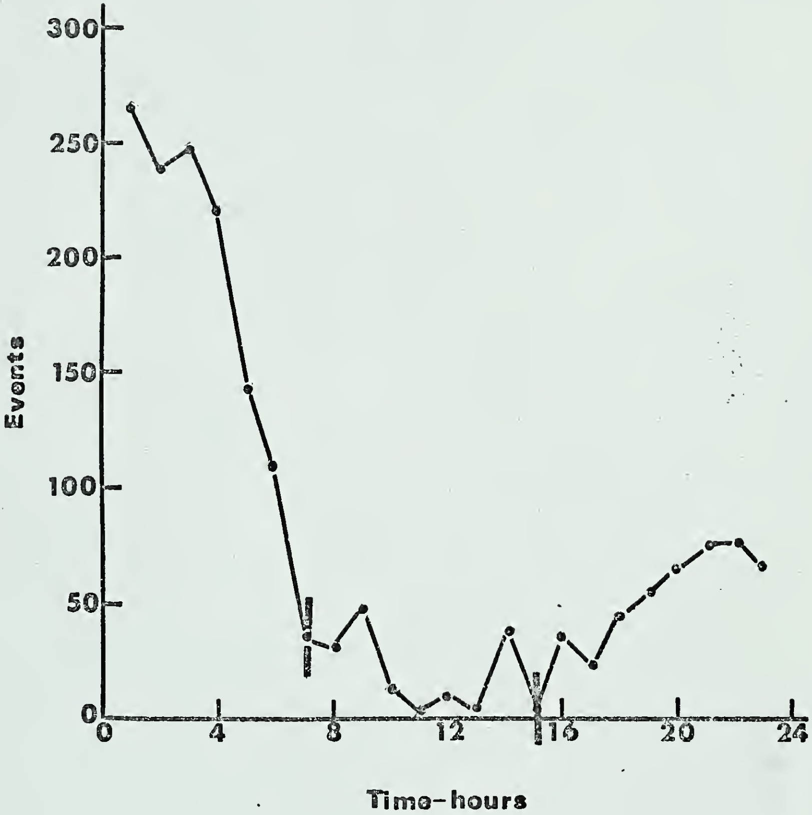
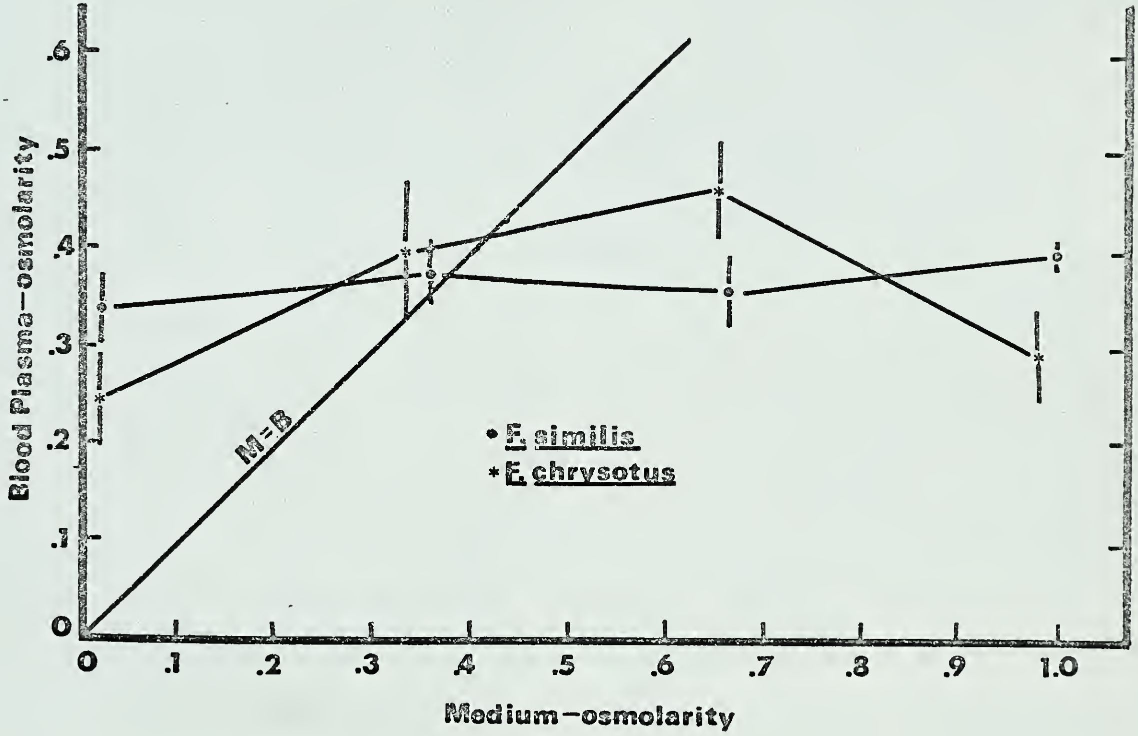

Events
300
250
200
150
100
50
0
0
4
8
12
16
20
24
Time-hours

Figure 4. Concentration of the blood plasma of both species as a function of the concentration of the medium. Two standard deviations are indicated around each mean.


Blood Plasma—osmolarity
0
.1
.2
.3
.4
.5
.6
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Medium—osmolarity
M=B
• E. similis
* E. chrysotus

is increased from 15 mosm/liter to 660 mosm/liter. At $[M] = 980$ mosm/liter, the blood plasma concentration drops to 297 ± 25.3 mosm/liter (Figure 4). The point of osmoneutrality is at $[M] = 405$ mosm/liter $= [B]$. All means are significantly different from one another $(P<.001)$.

Intact Animal Oxygen Consumption

There are significant variations in the oxygen consumption of intact *F. similis* in response to variations in salinity. The results are presented graphically in Figures 5 - 8 and are summarized in Table 1. The slopes of the log oxygen consumption-log body weight regressions are lowest in 1/3 sea water, 0.4916, and increase as $|B - M|$ increases. In fresh water, the slope is 0.6896, an increase of 40%. In 2/3 sea water, the slope increases 10% to 0.5432 and in sea water it is 0.5801, an increase of 30%. There are no significant difference among these slopes (Table 2).

The intercepts of these regression lines increase from -1.0655 in fresh water (0.086 ml/hr) to -0.8239 in full sea water (0.150 ml/hr). All intercepts are statistically different from each other, except those in 1/3 and 2/3 sea water (Table 2).

The slopes of the log oxygen consumption-log body weight regressions for *F. chrysotus* follow the same pattern as for *F. similis*. See Figures 9 - 12 and Table 3. The slopes are minimal in 1/3 sea water, 0.3430, and increase as $|B - M|$ increases. In fresh water, the slope is 0.3578, a 10% increase over the slope in 1/3 sea water. In 2/3 sea water, it increases 12% to 0.4119 and it is 0.5077, a 15% increase, in full sea water. Statistically, the slope in sea water

Figure 5. Oxygen consumption of intact *F. similis* in fresh water as a function of body weight. Slope = 0.6896 ± 0.0755. Intercept = -1.0655 ± 0.0436. Correlation coefficient = 0.91.

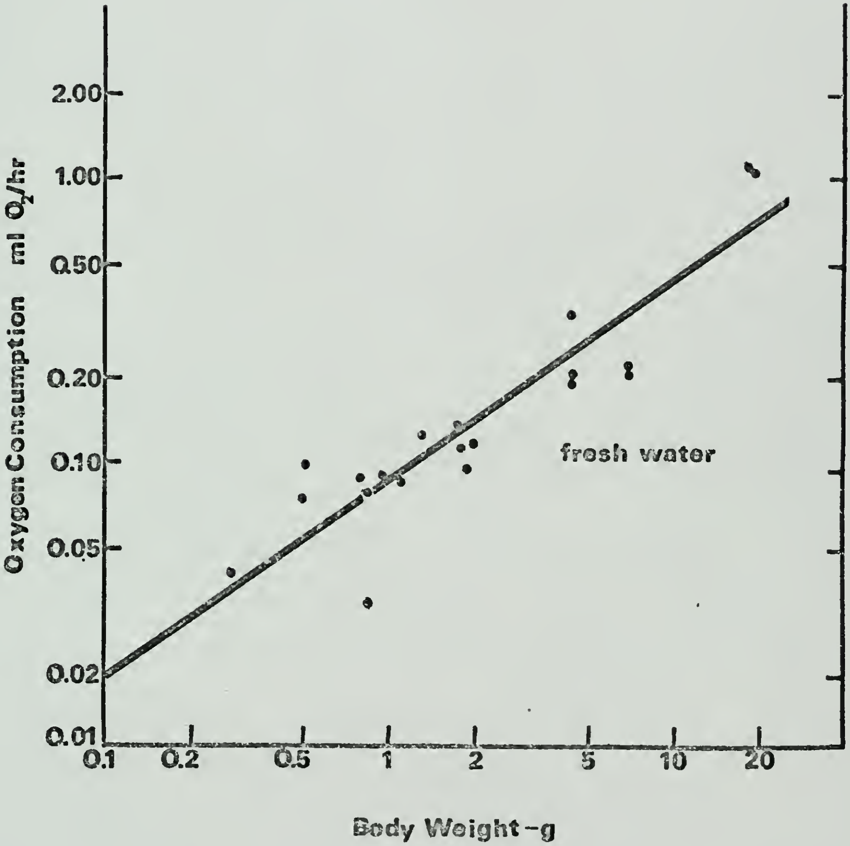

Oxygen Consumption ml O_2/hr
2.00
1.00
0.50
0.20
0.10
0.05
0.02
0.01
0.1
0.2
0.5
1
2
5
10
20
Body Weight-g
fresh water

Figure 6. Oxygen consumption of intact _F. similis_ in 1/3 sea water as a function of body weight. Slope = 0.4916 ± 0.0772. Intercept = -0.9508 ± 0.0274. Correlation coefficient = 0.81.

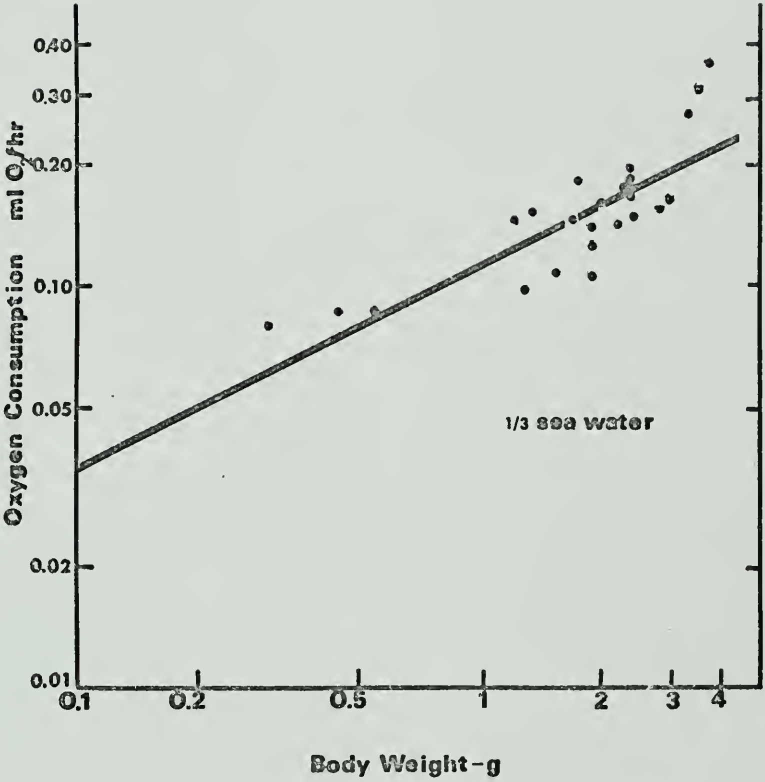

Oxygen Consumption ml O2/hr
0.40
0.30
0.20
0.10
0.05
0.02
0.01
0.1
0.2
0.5
1
2
3
4
Body Weight-g
1/3 sea water

Figure 7. Oxygen consumption of intact F. similis in 2/3 sea water as a function of body weight. Slope = 0.5432 ± 0.0927. Intercept = -0.9281 ± 0.0294. Correlation coefficient = 0.77.

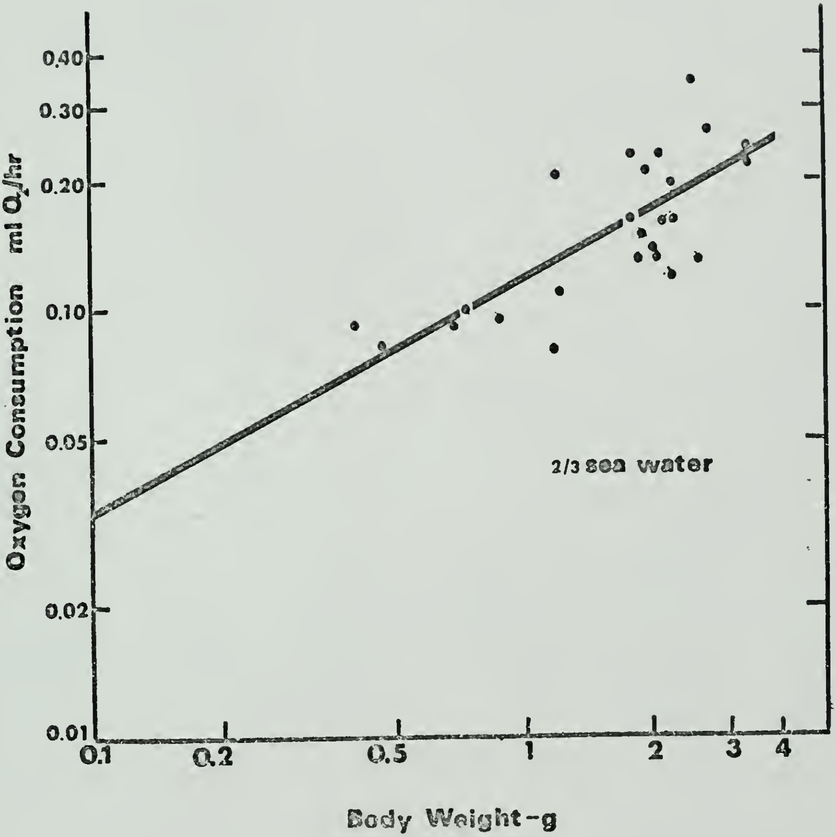

0.40
0.30
0.20
0.10
0.05
0.02
0.01
Oxygen Consumption ml O_2/hr
0.1
0.2
0.5
1
2
3
4
Body Weight-g
2/3 sea water

Figure 8. Oxygen consumption of intact *F. similis* in full sea water as a function of body weight. Slope = 0.5801 ± 0.0625. Intercept = -0.8239 ± 0.0469. Correlation coefficient = 0.89.

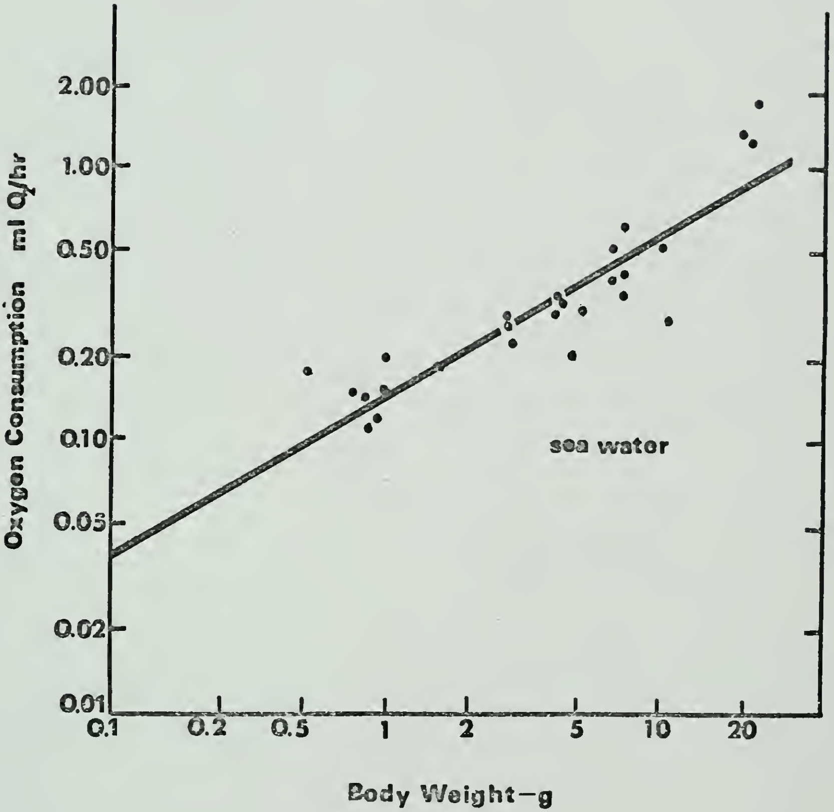

Oxygen Consumption ml O2/hr
2.00
1.00
0.50
0.20
0.10
0.05
0.02
0.01
0.1
0.2
0.5
1
2
5
10
20
sea water
Body Weight–g

TABLE 1

THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND WEIGHT OF INTACT *F. similis* AT FOUR SALINITIES

n	Concentration (mosmolar)	Salinity	Slope ± SE	Intercept ± SD	r	SD
20	25	f	0.6896 ± 0.0755	-1.0655 ± 0.0436	0.91	0.1676
24	350	1/3	0.4916 ± 0.0772	-0.9508 ± 0.0274	0.81	0.1004
25	667	2/3	0.5432 ± 0.0927	-0.9281 ± 0.0294	0.77	0.1118
25	1,000	3/3	0.5801 ± 0.0625	-0.8239 ± 0.0469	0.89	0.1435

TABLE 2

STATISTICAL COMPARISONS OF SLOPES AND INTERCEPTS OF THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND WEIGHT OF INTACT _F._ _similis_ AT FOUR SALINITIES

Comparison	Slopes	Intercepts
f-1/3	$P>.05$	*$P<.05$
f-2/3	$P>.05$	*$P<.01$
f-3/3	$P>.05$	*$P<.001$
1/3-2/3	$P>.05$	$P>.05$
1/3-3/3	$P>.05$	*$P<.01$
2/3-3/3	$P>.05$	*$P<.05$

*Considered to be statistically significant.

Figure 9. Oxygen consumption of intact *F. chrysotus* in fresh water as a function of body weight. Slope = 0.3578 ± 0.0348. Intercept = -0.9208 ± 0.0118. Correlation coefficient = 0.91.

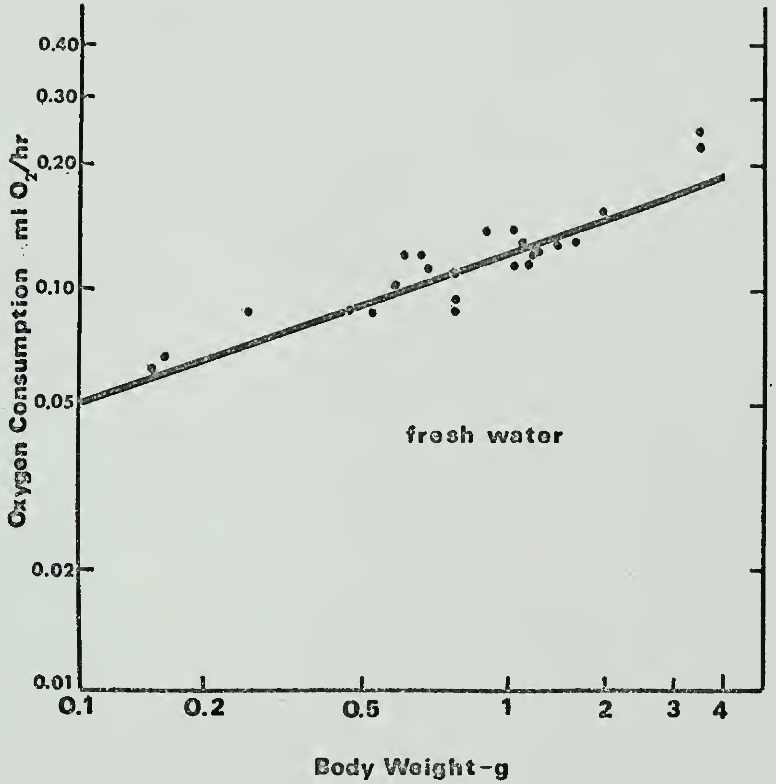

Oxygen Consumption ml O_2/hr
0.40
0.30
0.20
0.10
0.05
0.02
0.01
0.1
0.2
0.5
1
2
3
4
Body Weight-g
fresh water

Figure 10. Oxygen consumption of intact F. chrysotus in 1/3 sea water as a function of body weight. Slope = 0.3430 ± 0.0518. Intercept = -0.9066 ± 0.0148. Correlation coefficient = 0.81.

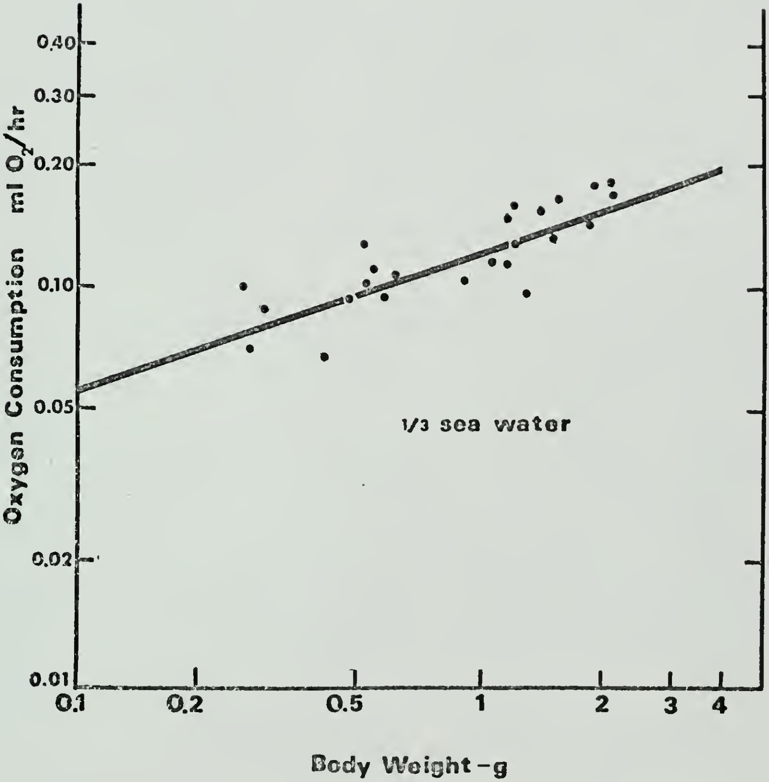

Oxygen Consumption ml O_2/hr
0.40
0.30
0.20
0.10
0.05
0.02
0.01
0.1
0.2
0.5
1
2
3
4
Body Weight – g
1/3 sea water

Figure 11. Oxygen consumption of intact *F. chrysotus* in 2/3 sea water as a function of body weight. Slope = 0.4119 ± 0.0511. Intercept = -0.9066 ± 0.0215. Correlation coefficient = 0.86.

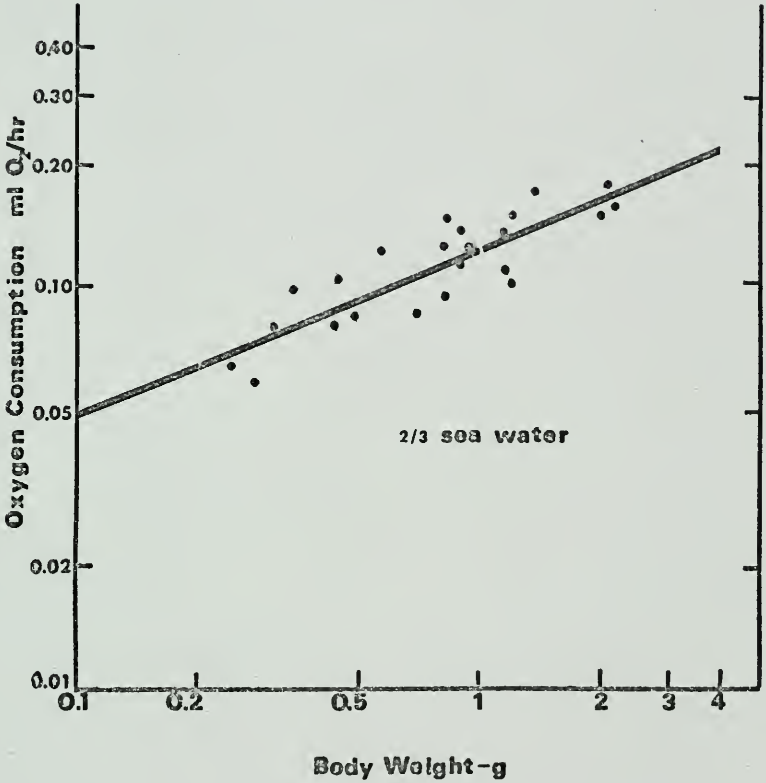

Oxygen Consumption ml O_2/hr
0.40
0.30
0.20
0.10
0.05
0.02
0.01
0.1
0.2
0.5
1
2
3
4
2/3 sea water
Body Weight-g

Figure 12. Oxygen consumption of intact *F. chrysotus* in full sea water as a function of body weight. Slope = 0.5077 ± 0.0424. Intercept = -1.0177 ± 0.0126. Correlation coefficient = 0.93.

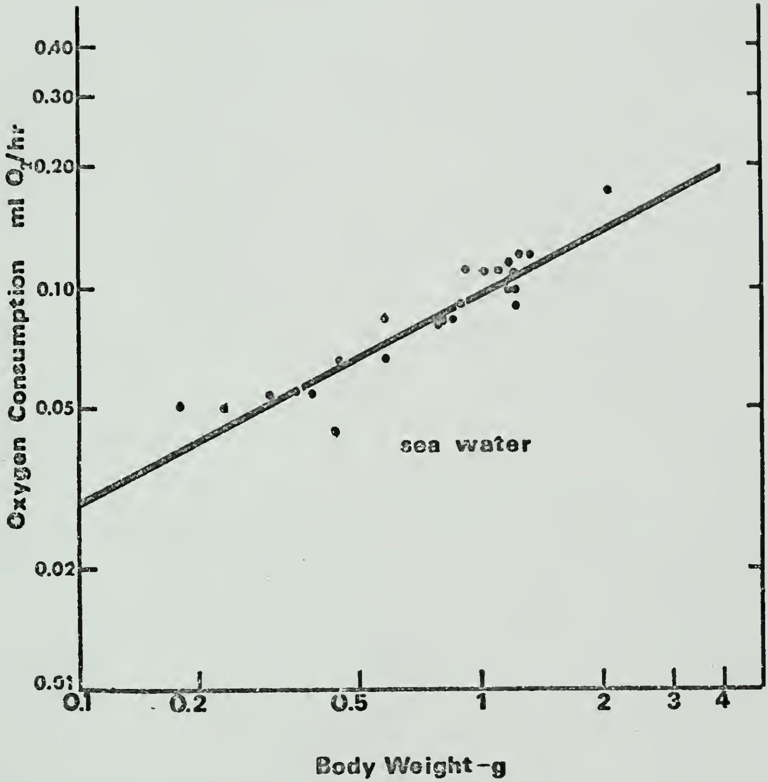

Oxygen Consumption ml O_2/hr
0.40
0.30
0.20
0.10
0.05
0.02
0.01
sea water
0.1
0.2
0.5
1
2
3
4
Body Weight-g

TABLE 3

THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND WEIGHT OF INTACT *F. chrysotus* AT FOUR SALINITIES

n	Concentration (mosmolar)	Salinity	Slope ± SE	Intercept ± SD	r	SD
24	15	f	0.3578 ± 0.0348	-0.9208 ± 0.0118	0.91	0.0574
24	330	1/3	0.3430 ± 0.0518	-0.9066 ± 0.0148	0.81	0.0692
24	660	2/3	0.4119 ± 0.0511	-0.9066 ± 0.0215	0.86	0.0670
24	980	3/3	0.5077 ± 0.0424	-1.0177 ± 0.0126	0.93	0.0556

differs from both the slope in fresh water ($P<.01$) and the slope in 1/3 sea water ($P<.05$). The hypothesis that any other differences in slopes exist is rejected ($P>.05$). See Table 4.

The intercepts of the regression lines for F. chrysotus do not show as much variation as those for F. similis. They are -0.9208 (0.120 ml/hr) in fresh water and -0.9066 (0.124 ml/hr) in 1/3 and 2/3 sea water. However, the intercept decreases in full sea water to -1.0177 (0.096 ml/hr), a result which is significantly different from all other intercepts ($P<.001$). See Table 4.

An interspecific comparison of slopes at each salinity shows that they differ in fresh water ($P<.001$). In all other salinities, a difference in slopes does not exist ($P>.05$). The intercepts differ in fresh and full sea water ($P<.001$), but do not differ in 1/3 and 2/3 sea water ($P>.05$). See Table 5.

Tables 6 and 7 show oxygen consumption of F. similis and F. chrysotus of five sizes at four different salinities. These values are calculated from the relationships given in Tables 1 and 3. Changes are calculated relative to values in 1/3 sea water which is nearest to being osmotically equivalent to the blood plasma.

Oxygen consumption is lowest in fresh water for F. similis weighing from 0.5 to 4.0 g. A 4.0 g fish has approximately the same oxygen consumption in fresh and 1/3 sea water. A 10.0 g fish shows a 21.1% increase in oxygen consumption in fresh water. In 2/3 sea water, fish of all sizes show increases in oxygen consumption (1.6% to 18.6%). In full sea water, increases range from 25.0% for a 0.5 g fish to 63.8% for a 10.0 g fish. All fish in a hyperosmotic medium show increases in oxygen consumption relative to 1/3 sea water. Small

TABLE 4

STATISTICAL COMPARISONS OF SLOPES AND INTERCEPTS OF THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND WEIGHT OF INTACT _F. chrysotus_ AT FOUR SALINITIES

Comparison	Slopes	Intercepts
f-1/3	$P > .05$	$P > .05$
f-2/3	$P > .05$	$P > .05$
f-3/3	*$P < .01$	*$P < .001$
1/3-2/3	$P > .05$	$P > .05$
1/3-3/3	*$P < .05$	*$P < .001$
2/3-3/3	$P > .05$	*$P < .001$

*Considered to be statistically significant.

TABLE 5

INTERSPECIFIC STATISTICAL COMPARISONS OF SLOPES AND INTERCEPTS OF THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND WEIGHT OF INTACT ANIMALS AT FOUR SALINITIES

Medium	Slopes	Intercepts
f	*$P<.001$	*$P<.001$
1/3	$P>.05$	$P>.05$
2/3	$P>.05$	$P>.05$
3/3	$P>.05$	*$P<.001$

*Considered to be statistically significant.

TABLE 6

OXYGEN CONSUMPTION OF INTACT *F. similis* AS A FUNCTION OF BODY SIZE AND SALINITY

Wt (g)	$\frac{\text{Surface area}}{\text{Wt}}$ (mm^2/g)	B-M = +314*		B-M = +25*		B-M = -317*		B-M = -603*	
		O_2 (ml/hr)	change %	O_2 (ml/hr)	change %	O_2 (ml/hr)	change %	O_2 (ml/hr)	change %
0.50	300	0.053	-33.9%	0.080	-	0.081	+ 1.6%	0.100	+25.0%
1.00	271	0.086	-23.2%	0.112	-	0.118	+ 5.4%	0.150	+33.9%
2.00	245	0.138	-12.6%	0.158	-	0.172	+ 9.2%	0.224	+41.7%
4.00	222	0.224	+ 0.9%	0.222	-	0.251	+13.1%	0.335	+50.6%
10.00	194	0.421	+21.1%	0.348	-	0.412	+18.6%	0.570	+63.8%

*Difference between medium concentration and blood plasma concentration in milliosmoles/liter.

TABLE 7

OXYGEN CONSUMPTION OF INTACT F. chrysotus AS A FUNCTION OF BODY SIZE AND SALINITY

Wt (g)	Surface area / Wt (mm^2/g)	B-M = +231*		B-M = +66*		B-M = -201*		B-M = -683*	
		O_2 (ml/hr)	change %	O_2 (ml/hr)	change %	O_2 (ml/hr)	change %	O_2 (ml/hr)	change %
0.50	204	0.094	-4.2%	0.098	-	0.093	-4.0%	0.068	-30.6%
0.75	219	0.108	-3.6%	0.112	-	0.110	-1.9%	0.083	-26.0%
1.00	230	0.120	-3.3%	0.124	-	0.124	0.0%	0.096	-22.6%
1.50	247	0.139	-2.8%	0.143	-	0.147	+2.8%	0.118	-17.5%
2.00	260	0.154	-2.2%	0.157	-	0.165	+5.0%	0.137	-12.7%

*Difference between medium concentration and blood plasma concentration in milliosmoles/liter.

fish (less than 4.0 g) in a hypoosmotic medium show decreases in oxygen consumption,while large fish (more than 4.0 g) in the same salinity show increases in oxygen consumption.

The oxygen consumption of *F. chrysotus* is relatively stable from fresh to 2/3 sea water and then drops in full sea water. In fresh water, all fish show slight decreases in oxygen consumption ranging from 4.2% for a 0.5 g fish to 2.2% for a 2.0 g fish. In 2/3 sea water, fish below 1.0 g show a slight decrease in oxygen consumption,and fish above 1.0 g show a slight increase in oxygen consumption. In full sea water, all fish show decreased oxygen consumptions with the greatest depressions occurring in the smallest fish.

The slopes obtained for the double logaritmic relationship between oxygen consumption and body weight are lower for both species in all salinities than those reported for other fishes. Job (1955) found slopes of 0.802 - 0.856 for *Salvelinus fontinalis*. Hickman (1959) found a slope of 0.86 to represent the effect of weight on the oxygen consumption of the starry flounder, *Platichthys stellatus*. He also found slope values of 0.84 for lemon sole, *Parophys vetulus*, and 0.90 for sanddab, *Citharichthys stigmaeus*. O'Hara (1968) found slopes of 0.717 and 0.710 for *Lepomis machrochirus* and *Lepomis gibbosus* respectively. Beamish and Mookherjii (1964) found an average slope of 0.85 for *Carassius auratus*. Only the value inferred from data of Wells (1935) for *Fundulus parvipinnis* approaches the values in this study. His data imply a slope of 0.50. All other studies concerning the dependence of oxygen consumption on weight in fishes have been conducted using larger fish than those in this study and only data of Wells are for fish of a comparable size range.

Oxygen Consumption of Excised Gills

The slopes of the regression lines of log oxygen consumption-log dry gill weight for F. similis are maximal (0.5817) in 1/3 sea water and decrease as |B - M| increases (Figures 13 - 16 and Table 8). In fresh water, the slope decreases 46% to 0.3151. In 2/3 sea water, it is 0.4144, a 29% decrease. In full sea water, the slope is minimal at 0.2453, a 57% decrease. Statistically, the slope in full sea water differs from both the slope in 1/3 sea water ($P<.001$) and the slope in 2/3 sea water ($P<.05$). See Table 9.

The intercepts of these regression lines for F. similis are minimal in 1/3 sea water (0.4751) and increase as |B - M| increases. In fresh water, the intercept increases 50% to 0.7121. Intercepts are 0.7526 and 0.9531 in 2/3 and 3/3 sea water respectively. These correspond to increases of 57% and 101% over the intercept in 1/3 sea water. The intercept in 1/3 sea water differs from the intercept in 2/3 sea water ($P<.02$) and from the intercept in full sea water ($P<.001$). See Table 9.

The gills of F. chrysotus were more difficult to handle because they were smaller (1 - 20 mg dry weight) than gills of F. similis (1 - 157 mg dry weight). Only 55% (66 of 120) of the excised gills survived the operation and consumed oxygen when placed in the respirometers. Therefore, the following results are of questionable value.

The slopes of the regression of log oxygen consumption-log dry gill weight for F. chrysotus vary neither directly nor indirectly with |B - M| (Figures 17 - 20 and Table 10). The slope is minimal in

Figure 13. Oxygen consumption of excised gills of F. similis in fresh water as a function of dry weight of gills. Slope = 0.3151 ± 0.1311. Intercept = 0.7121 ± 0.1325. Correlation coefficient = 0.47.

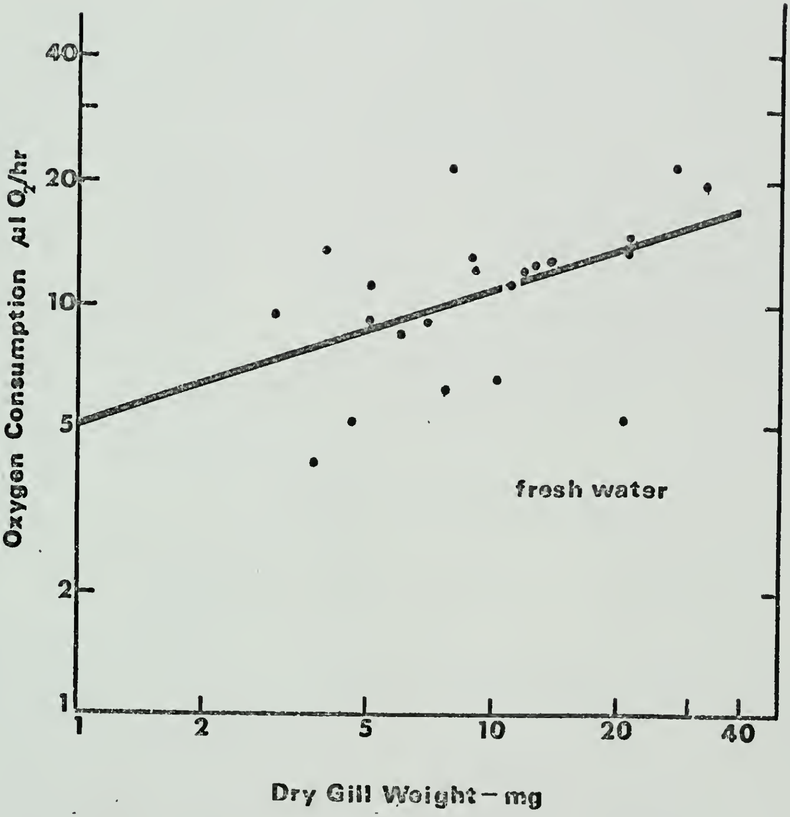

Oxygen Consumption µl O_2/hr
40
20
10
5
2
1
1
2
5
10
20
40
fresh water
Dry Gill Weight – mg

Figure 14. Oxygen consumption of excised gills of F. *similis* in 1/3 sea water as a function of dry weight of gills. Slope = 0.5817 ± 0.0673. Intercept = 0.4751 ± 0.0685. Correlation coefficient = 0.87.

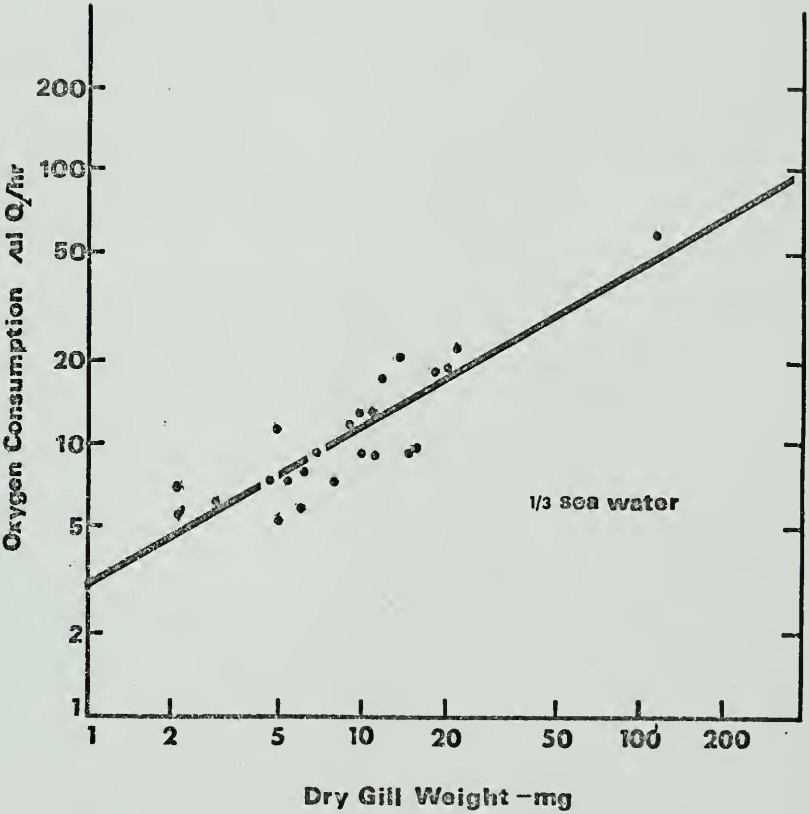

Oxygen Consumption µl O₂/hr
200
100
50
20
10
5
2
1
1
2
5
10
20
50
100
200
Dry Gill Weight –mg
1/3 sea water

Figure 15. Oxygen consumption of excised gills of F. similis in 2/3 sea water as a function of dry weight of gills. Slope = 0.4144 ± 0.0764. Intercept = 0.7526 ± 0.0888. Correlation coefficient = 0.77.

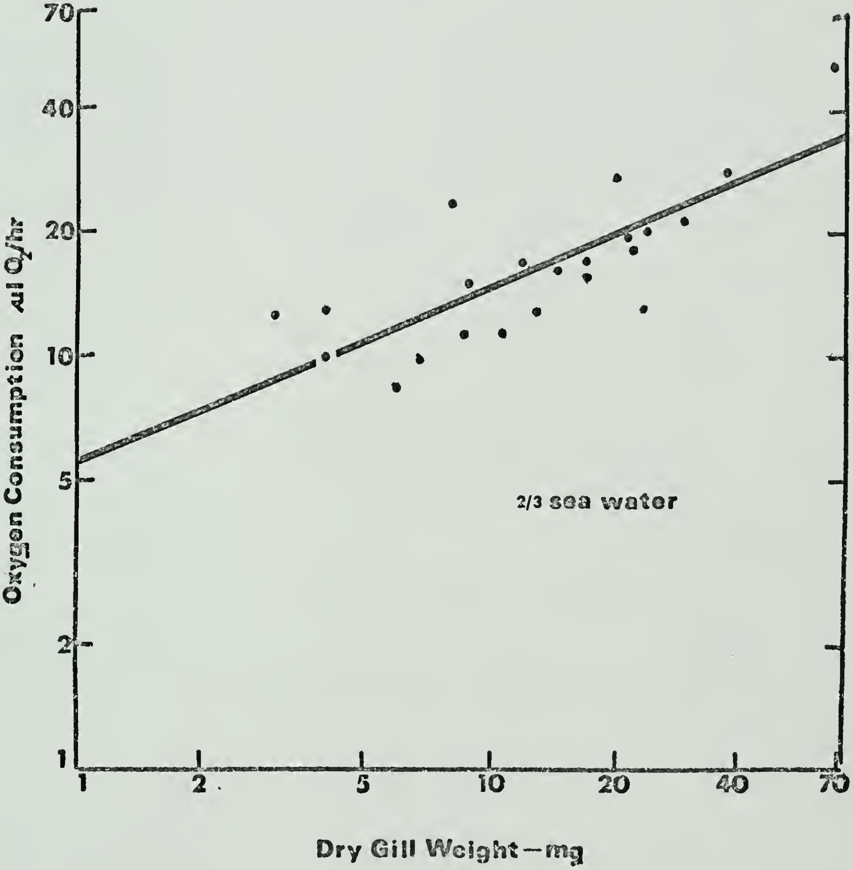

70
40
20
10
5
2
1
Oxygen Consumption µl O2/hr
2/3 sea water
1
2
5
10
20
40
70
Dry Gill Weight—mg

Figure 16. Oxygen consumption of excised gills of _F_. _similis_ in full sea water as a function of dry weight of gills. Slope = 0.2453 ± 0.0592. Intercept = 0.9531 ± 0.0685. Correlation coefficient = 0.66.

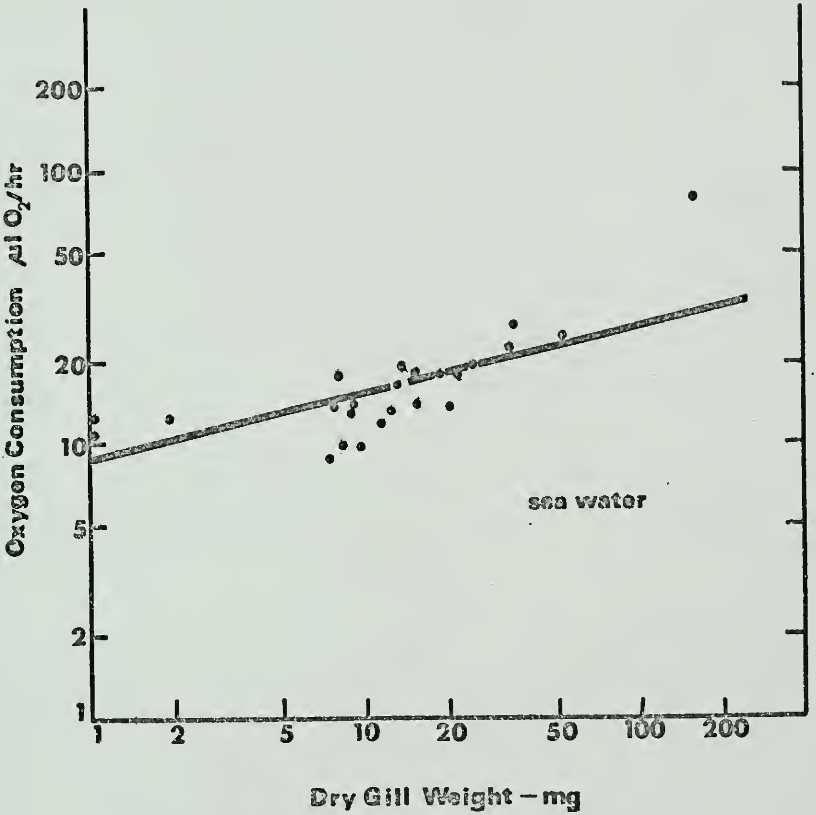

Oxygen Consumption µl O₂/hr
200
100
50
20
10
5
2
1
1
2
5
10
20
50
100
200
Dry Gill Weight — mg
sea water

TABLE 8

THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN COMSUMPTION AND DRY WEIGHT OF EXCISED GILLS OF F. similis AT FOUR SALINITIES

n	Concentration (mosmolar)	Salinity	Slope ± SE	Intercept ± SD	r	SD
22	25	f	0.3151 ± 0.1311	0.7121 ± 0.1325	0.47	0.1760
24	350	1/3	0.5817 ± 0.0673	0.4751 ± 0.0685	0.87	0.1195
22	667	2/3	0.4144 ± 0.0764	0.7526 ± 0.0888	0.77	0.1187
24	1,000	3/3	0.2453 ± 0.0592	0.9531 ± 0.0685	0.66	0.1486

TABLE 9

STATISTICAL COMPARISONS OF SLOPES AND INTERCEPTS OF THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND DRY WEIGHT OF EXCISED GILLS OF *F. similis* AT FOUR SALINITIES

Comparison	Slopes	Intercepts
f-1/3	$P > .05$	$P > .05$
f-2/3	$P > .05$	$P > .05$
f-3/3	$P > .05$	$P > .05$
1/3-2/3	$P > .05$	*$P < .02$
1/3-3/3	*$P < .001$	*$P < .001$
2/3-3/3	*$P < .05$	$P > .05$

*Considered to be statistically significant.

Figure 17. Oxygen consumption of excised gills of F. *chrysotus* in fresh water as a function of dry weight of gills. Slope = 0.3436 ± 0.1216. Intercept = 0.6022 ± 0.0801. Correlation coefficient = 0.60.

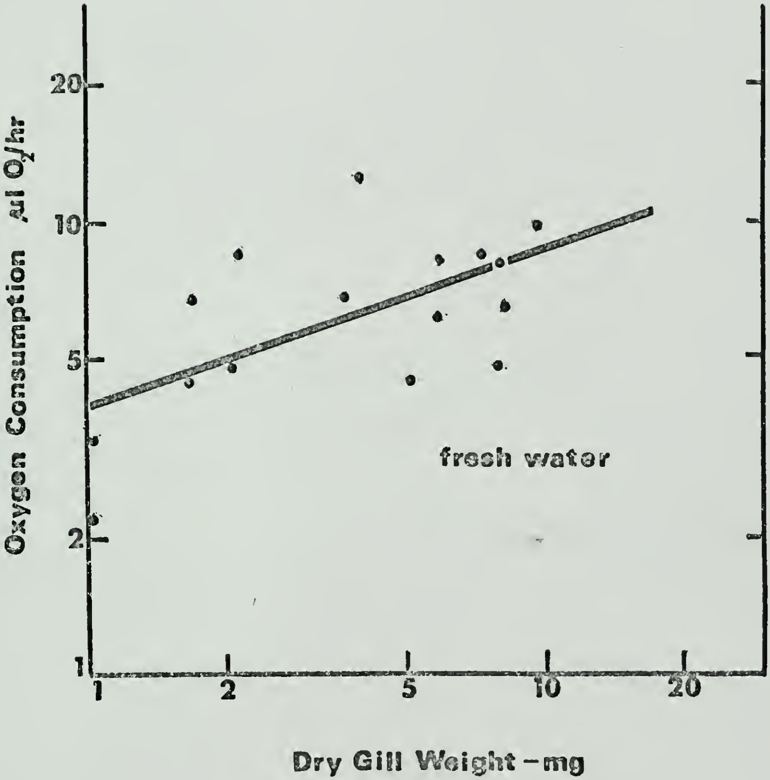

Oxygen Consumption µl O₂/hr
20
10
5
2
1
1
2
5
10
20
fresh water
Dry Gill Weight – mg

Figure 18. Oxygen consumption of excised gills of F. chrysotus in 1/3 sea water as a function of dry weight of gills. Slope = 0.1383 ± 0.3402. Intercept = 0.7599 ± 0.1991. Correlation coefficient = 0.11.

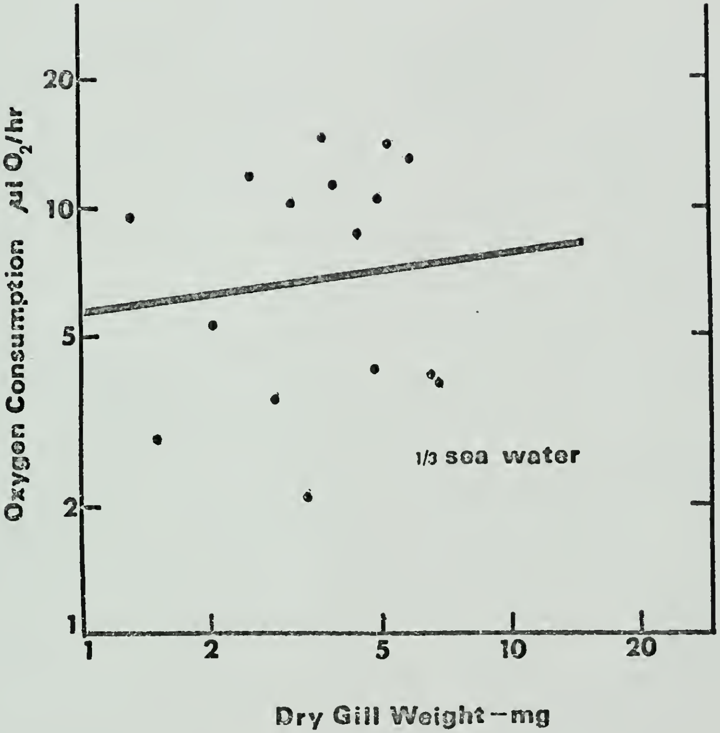

Oxygen Consumption μl O_2/hr
20
10
5
2
1
1
2
5
10
20
1/3 sea water
Dry Gill Weight—mg

Figure 19. Oxygen consumption of excised gills of F. chrysotus in 2/3 sea water as a function of dry weight of gills. Slope = 0.5173 ± 0.1845. Intercept = 0.5533 ± 0.0979. Correlation coefficient = 0.59.

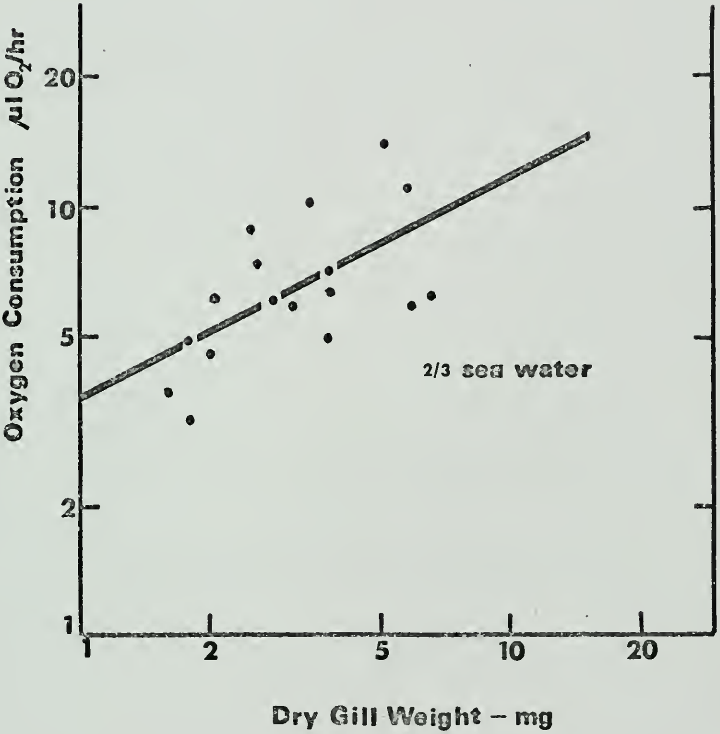

Oxygen Consumption μl O$_2$/hr
20
10
5
2
1
1
2
5
10
20
Dry Gill Weight – mg
2/3 sea water

Figure 20. Oxygen consumption of excised gills of _F. chrysotus_ in full sea water as a function of dry weight of gills. Slope = 0.4584 ± 0.1315. Intercept = 0.6302 ± 0.0937. Correlation coefficient = 0.67.

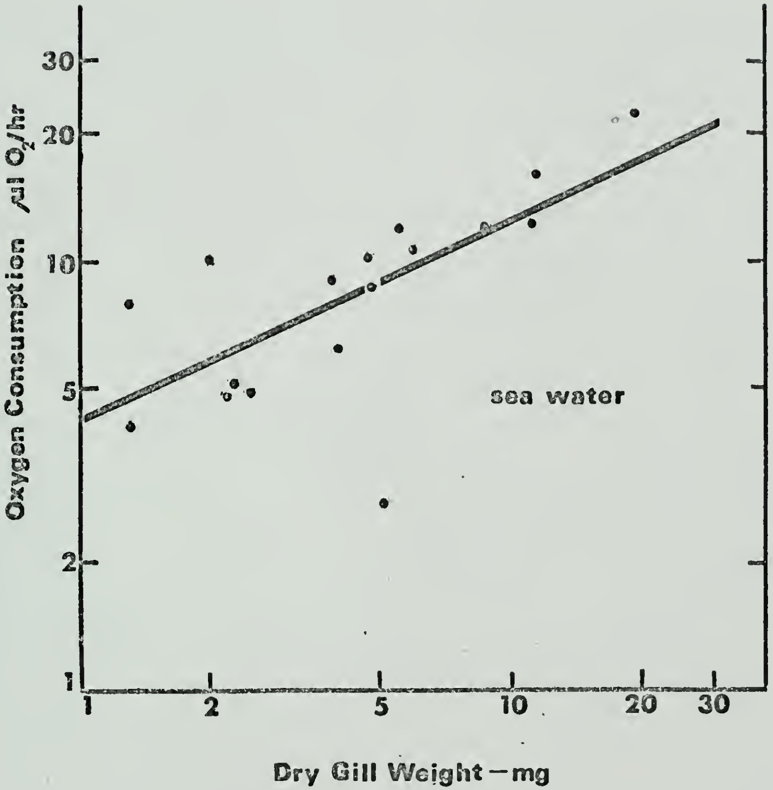

Oxygen Consumption µl O$_2$/hr
30
20
10
5
2
1
1
2
5
10
20
30
Dry Gill Weight – mg
sea water

TABLE 10

THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND DRY WEIGHT OF EXCISED GILLS OF *F. chrysotus* AT FOUR SALINITIES

n	Concentration (mosmolar)	Salinity	Slope ± SE	Intercept ± SD	r	SD
16	15	f	0.3436 ± 0.1216	0.6022 ± 0.0801	0.60	0.1593
16	330	1/3	0.1383 ± 0.3402	0.7599 ± 0.1991	0.11	0.2840
17	660	2/3	0.5173 ± 0.1845	0.5533 ± 0.0979	0.59	0.1435
17	980	3/3	0.4584 ± 0.1315	0.6302 ± 0.0937	0.67	0.1767

1/3 sea water, 0.1383. In fresh water, it increases 148% to 0.3436. In 2/3 sea water, the slope is 0.5173, a 274% increase. However, in full sea water the slope drops to 0.4584, a 231% increase over the slope in 1/3 sea water. There are no statistical differences among these slopes (Table 11).

The intercept of this relationship for F. chrysotus is minimal in 2/3 sea water, 0.5533. It increases 9% to 0.6022 in fresh water. In 1/3 sea water the slope is maximal at 0.7599, an increase of 38% over the slope in 2/3 sea water. The intercept for the relationship in full sea water is 0.6302, 5% greater than the intercept for the relationship in 2/3 sea water. Statistically, none of these intercepts differs from each other (Table 11).

Comparing slopes and intercepts at each salinity for the two species shows that the intercepts differ in sea water ($P<.01$). See Table 12.

Tables 13 and 14 show the amount of oxygen consumed by excised gills of various sizes of fish in four different salinities. Gill weight is calculated for each size fish based on the relationships in Table 18. Oxygen consumption is derived from the relationships in Tables 8 and 10. For a small F. similis (0.5 g), oxygen consumption of the gills increases as $|B - M|$ increases. A 1.0 g fish also shows increases relative to 1/3 sea water when it is in a hyperosmotic medium, but there is no rise in gill oxygen consumption when it is in a hypoosmotic medium. Fish above 1.0 g show decreases in gill oxygen consumption in fresh water. Fish up to ca. 4.0 g show increases in gill oxygen consumption during hypoosmotic regulation. Above this weight, decreases in gill oxygen consumption occur and

TABLE 11

STATISTICAL COMPARISONS OF SLOPES AND INTERCEPTS OF THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND DRY WEIGHT OF EXCISED GILLS OF *F. chrysotus* AT FOUR SALINITIES

Comparison	Slopes	Intercepts
f-1/3	$P > .05$	$P > .05$
f-2/3	$P > .05$	$P > .05$
f-3/3	$P > .05$	$P > .05$
1/3-2/3	$P > .05$	$P > .05$
1/3-3/3	$P > .05$	$P > .05$
2/3-3/3	$P > .05$	$P > .05$

*Considered to be statistically significant.

TABLE 12

INTERSPECIFIC STATISTICAL COMPARISONS OF SLOPES AND INTERCEPTS OF THE DOUBLE LOGARITHMIC RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND DRY WEIGHT OF EXCISED GILLS AT FOUR SALINITIES

Medium	Slopes	Intercepts
f	$P>.05$	$P>.05$
1/3	$P>.05$	$P>.05$
2/3	$P>.05$	$P>.05$
3/3	$P>.05$	*$P<.01$

*Considered to be statistically significant.

TABLE 13

OXYGEN CONSUMPTION OF EXCISED GILLS OF *F. similis* AS A FUNCTION OF BODY SIZE AND SALINITY

Wt (g)	Gill wt (mg)	B-M = +314*		B-M = +25*		B-M = -317*		B-M = -603*	
		O_2 (µl/hr)	change %	O_2 (µl/hr)	change %	O_2 (µl/hr)	change %	O_2 (µl/hr)	change %
0.50	3.95	7.94	+19.5%	6.64	-	10.00	+50.0%	12.56	+89.0%
1.00	7.83	9.86	0.0%	9.87	-	13.27	+34.0%	14.86	+51.0%
2.00	15.52	12.22	-17.0%	14.72	-	17.62	+19.0%	17.58	+19.0%
4.00	30.83	15.17	-31.0%	21.93	-	23.39	+6.6%	20.80	-5.2%
10.00	76.03	20.18	-46.0%	37.15	-	34.04	-8.4%	26.00	-30.1%

* Difference between medium concentration and blood plasma concentration in milliosmoles/liter.

TABLE 14

OXYGEN CONSUMPTION OF EXCISED GILLS OF _F. chrysotus_ AS A FUNCTION OF BODY SIZE AND SALINITY

		B-M = +231*		B-M = +66*		B-M = -201*		B-M = -683*	
Wt (g)	Gill wt (mg)	O_2 (µl/hr)	change %	O_2 (µl/hr)	change %	O_2 (µl/hr)	change %	O_2 (µl/hr)	change %
0.50	2.58	5.53	-15.7%	6.56	-	5.83	-11.2%	6.59	+0.5%
0.75	3.81	6.34	-8.4%	6.92	-	7.15	+3.3%	7.89	+14.0%
1.00	5.04	6.97	-3.1%	7.19	-	8.24	+14.6%	8.95	+24.5%
1.50	7.45	7.98	+5.0%	7.60	-	10.10	+32.9%	10.72	+41.1%
2.00	9.84	8.77	+11.2%	7.89	-	11.67	+47.9%	12.16	+54.1%

*Difference between medium concentration and blood plasma concentration in milliosmoles/liter.

become greater as $|B - M|$ increases. The results for *F. chrysotus* show increases in gill oxygen consumption relative to 1/3 sea water for all fish in full sea water and for fish larger than 0.5 g in 2/3 sea water. A 0.5 g fish shows a decreased gill oxygen consumption in 2/3 sea water. Fish weighing 1.0 g or less have depressed oxygen consumptions in fresh water. Fish weighing 1.5 g or 2.0 g show increases in gill oxygen consumption in fresh water.

Oxygen consumption of excised gills increases less than proportionately as dry gill weight increases. Slopes range from 0.2453 to 0.5817 for *F. similis* and from 0.1383 to 0.5173 for *F. chrysotus*. Studies of Weymouth *et al.* (1944), Bertalanffy and Perozynski (1951, 1953) and Vernberg (1954, 1956) state that, at least in some tissues, weight specific metabolic rate decreases as body size increases. More specifically, studies with gill tissue by Dehnel and McCanghran (1964) and Holmes and Stott (1960) have indicated slopes of 0.83 and 0.86 respectively. These are considerably greater than the slopes found in this study.

Comparison of Variations in Oxygen Consumption of Intact Animals and Excised Gills

Changes in oxygen consumption of intact animals do not parallel changes in oxygen consumption of excised gills as a function of salinity (Table 15). In fresh water, intact *F. similis* shows reductions in oxygen consumption of animals weighing less than 4.0 g. Animals below 1.0 g show increased gill oxygen consumptions, but animals above 1.0 g show decreased gill oxygen consumptions. In 2/3 sea water, increases in the oxygen consumption of intact animals are

TABLE 15

COMPARISON OF PERCENTAGE CHANGE IN OXYGEN CONSUMPTION OF INTACT ANIMALS AND EXCISED GILLS AS A FUNCTION OF SALINITY

	Fundulus similis			*Fundulus chrysotus*	
Wt (g)	Intact animal*	Excised gill*	Wt (g)	Intact animal*	Excised gill*
	fresh water			fresh water	
0.50	-33.9%	+19.5%	0.50	-4.2%	-15.7%
1.00	-23.2%	0.0%	0.75	-3.6%	-8.4%
2.00	-12.6%	-17.0%	1.00	-3.3%	-3.1%
4.00	+0.9%	-31.0%	1.50	-2.8%	+5.0%
10.00	+21.1%	-46.0%	2.00	-2.2%	+11.2%
	2/3 sea water			2/3 sea water	
0.50	+1.6%	+50.0%	0.50	-4.0%	-11.2%
1.00	+5.4%	+34.0%	0.75	-1.9%	+3.3%
2.00	+9.2%	+19.0%	1.00	0.0%	+14.6%
4.00	+13.1%	+6.6%	1.50	+2.8%	+32.9%
10.00	+18.6%	-8.4%	2.00	+5.0%	+47.9%
	3/3 sea water			3/3 sea water	
0.50	+25.0%	+89.0%	0.50	-30.6%	+0.5%
1.00	+33.9%	+51.0%	0.75	-26.0%	+14.0%
2.00	+41.7%	+19.0%	1.00	-22.6%	+24.5%
4.00	+50.6%	-5.2%	1.50	-17.5%	+41.1%
10.00	+63.8%	-30.1%	2.00	-12.7%	+54.1%

*Data are given as a percentage change in oxygen consumption relative to oxygen consumption in 1/3 sea water.

present in all sizes of fish and the percentage increase becomes larger as body size increases. Oxygen consumption of excised gills also shows increases, but the percentage decreases with increasing body size, until at 10.0 g there is an 8.4% reduction in gill oxygen consumption. In 3/3 sea water, all F. similis show increased oxygen consumption by intact animals and the percentage change relative to 1/3 sea water increases with increasing body size. Fish weighing 2.0 g or less show increased gill oxygen consumption, but 4.0 and 10.0 g fish show decreased gill oxygen consumption.

All sizes of F. chrysotus show decreased oxygen consumption of intact animals in fresh water. Animals weighing 1.0 g or less also show decreased gill oxygen consumption. However, animals weighing either 1.5 or 2.0 g show increases in gill oxygen consumption. In 2/3 sea water, oxygen consumption of intact animals decreases for animals of less than 1.0 g and increases for animals weighing more than 1.0 g. Oxygen consumption of gills of animals of all sizes except 0.50 g shows increases. In 3/3 sea water, oxygen consumption of intact animals of all sizes is reduced, while gill oxygen consumption of all animals is elevated.

There is no apparent correlation between changes in oxygen consumption of intact animals and changes in oxygen consumption of excised gills for either of the species.

Apparent Permeability

Apparent permeabilities which have been calculated with the equation of Potts (1954) are given in Tables 16 and 17. In all

TABLE 16

APPARENT PERMEABILITY OF *F. similis* AS A FUNCTION OF BODY SIZE AND SALINITY

		B-M = +314#		B-M = +25#
Wt (g)	Surface area (mm^2)	Permeability* (M/mm^2-M-hr)	change %	Permeability* (M/mm^2-M-hr)
0.50	150	4.11×10^{-6}	-99.0%	3.84×10^{-4}
1.00	251	2.82×10^{-6}	-99.1%	3.17×10^{-4}
2.00	490	1.93×10^{-6}	-99.3%	2.61×10^{-4}
4.00	887	1.32×10^{-6}	-99.4%	2.51×10^{-4}
10.00	1,941	0.80×10^{-6}	-99.5%	1.66×10^{-4}

#Difference between the medium concentration and blood plasma concentration in milliosmoles/liter.

*Moles per square millimeter per molar concentration difference per hour.

TABLE 16 (Extended)

	B-M = -317[#]		B-M = -603[#]	
change %	Permeability[*] (M/mm^2-M-hr)	change %	Permeability[*] (M/mm^2-M-hr)	change %
-	5.06 X 10^{-6}	-98.7%	2.23 X 10^{-6}	-99.4%
-	3.72 X 10^{-6}	-98.8%	1.46 X 10^{-6}	-99.5%
-	2.73 X 10^{-6}	-99.0%	0.95 X 10^{-6}	-99.6%
-	2.00 X 10^{-6}	-99.1%	0.62 X 10^{-6}	-99.7%
-	1.33 X 10^{-6}	-99.25	0.35 X 10^{-6}	-99.8%

TABLE 17

APPARENT PERMEABILITY OF *F. chrysotus* AS A FUNCTION OF BODY SIZE AND SALINITY

		B-M = +231[#]		B-M = +66[#]
Wt (g)	Surface area (mm^2)	Permeability[*] (M/mm^2-M-hr)	change %	Permeability[*] (M/mm^2-M-hr)
0.50	102	4.36×10^{-6}	94.5%	7.86×10^{-5}
0.75	164	3.11×10^{-6}	94.0%	5.14×10^{-5}
1.00	230	2.45×10^{-6}	93.6%	3.82×10^{-5}
1.50	371	1.73×10^{-6}	93.1%	2.51×10^{-5}
2.00	520	1.35×10^{-6}	92.7%	1.85×10^{-5}

[#] Difference between the medium concentration and blood plasma concentration in milliosmoles/liter.

[*] Moles per square millimeter per molar concentration difference per hour.

TABLE 17 (Extended)

	B-M = -201[#]		B-M = -683[#]	
change %	Permeability[*] (M/mm^2-M-hr)	change %	Permeability[*] (M/mm^2-M-hr)	change %
-	1.16×10^{-5}	85.3%	1.17×10^{-6}	98.5%
-	8.79×10^{-6}	82.9%	8.73×10^{-7}	98.3%
-	7.25×10^{-6}	81.0%	7.05×10^{-7}	98.2%
-	5.51×10^{-6}	78.0%	5.26×10^{-7}	98.0%
-	4.53×10^{-6}	75.5%	4.25×10^{-7}	97.7%

salinities for both species, the smallest fish have the greatest permeabilities. Percentage decreases relative to permeabilities in 1/3 sea water are also given in Tables 16 and 17. The percentage decrease from 1/3 sea water increases with body size in all salinities for _F. similis_. For _F. chrysotus_, this factor decreases with body size in all salinities.

Anatomical Relationships

The log gill surface area-log body weight relationship for _F. similis_ indicates that gill surface area increases less than proportionately as body weight increases (Figure 21 and Table 18). The slope equals 0.8457 and the intercept is 2,4330. A similar analysis for _F. chrysotus_ shows that gill surface area increases more than proportionately as body weight increases. The slope equals 1.1753 and the intercept is 2.3623 (Figure 22 and Table 18). The slopes differ statistically from each other, as do the intercepts with $P<.01$ in both cases (Table 24).

The most notable studies concerning gill surface area of fishes are by Gray (1954), Steen and Kruysse (1964), and Hughes (1966). Gray (1954) found surface areas ranging from 188 mm^2/g for _Lophopsetta maculatus_ to 1773 mm^2/g for _Brevoortia tyrannus_. Steen and Kruysse (1964) found that a 550 g eel had 530 cm^2 of gill surface area or 1,075 mm^2/g. Hughes (1966) found surface areas ranging from 168 mm^2/g for a 24 g _Callionymlus lyra_ to 845 mm^2/g for an 85 g _Clupea harengus_. The surface area of _F. similis_ ranges from 154.3 mm^2/g for a 9.25 g fish to 313.4 mm^2/g for a 1.46 g fish. The surface area of _F. chrysotus_ ranges from 148.6 mm^2/g for a 0.36 g fish to 279.7 mm^2/g for a 3.88 g fish.

Figure 21. Surface area of gills of F. *similis* as a function of body weight. Slope = 0.8457 ± 0.0307. Intercept = 2.4330 ± 0.0204. Correlation coefficient = 0.99.

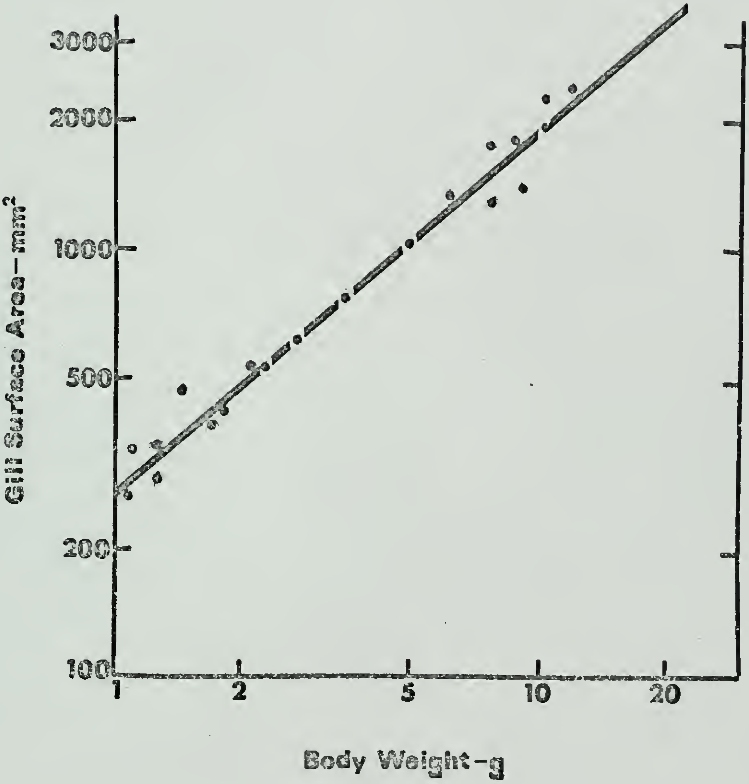

Gill Surface Area – mm^2
3000
2000
1000
500
200
100
1
2
5
10
20
Body Weight – g

Figure 22. Surface area of gills of _F. chrysotus_ as a function of body weight. Slope = 1.1753 ± 0.0294. Intercept = 2.3623 ± 0.0133. Correlation coefficient = 0.99.

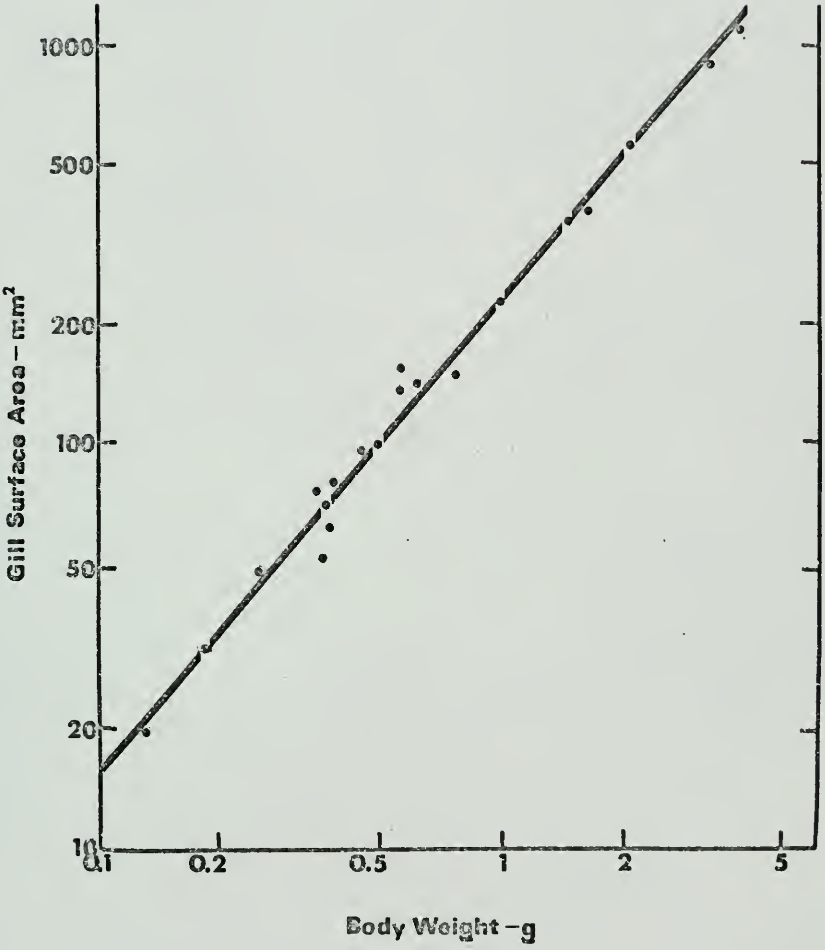

Gill Surface Area — mm²
1000
500
200
100
50
20
10
0.1
0.2
0.5
1
2
5
Body Weight — g

TABLE 18

ANATOMICAL RELATIONSHIPS

Relationship	n	Slope ± SE	Intercept ± SD	r	SD
Surface area-Body wt*					
F. similis	20	0.8457 ± 0.0307	2.4330 ± 0.0204	0.99	0.0500
F. chrysotus	20	1.1753 ± 0.0294	2.3623 ± 0.0133	0.99	0.0509
Dry gill wt-Body wt*					
F. similis	92	0.9874 ± 0.0545	0.8940 ± 0.0201	0.89	0.1783
F. chrysotus	66	0.9671 ± 0.1022	0.7021 ± 0.0284	0.65	0.2814

*Double logarithmic relationship

TABLE 19

INTERSPECIFIC COMPARISON OF SLOPES AND INTERCEPTS OF ANATOMICAL RELATIONSHIPS

Relationship	Slopes	Intercepts
Surface area-Body wt#	*$P < .01$	*$P < .01$
Dry gill wt-Body wt#	$P > .05$	*$P < .001$

Double logarithmic relationship.

* Considered to be statistically significant.

Only Price (1931) has previously studied the gill surface area of a fish as a function of body weight and has found a slope of 0.78 for *Micropterus dolomieu*. This is lower than the slopes of 0.846 for *F. similis* and 1.175 for *F. chrysotus*.

Dry gill weight increases proportionately with body weight in both *F. similis* and *F. chrysotus* (Figures 23 and 24). The slopes are 0.9874 for *F. similis* and 0.9671 for *F. chrysotus*. These are not significantly different from each other ($P>.05$). The intercepts of this relationship are 0.8940 for *F. similis* and 0.7021 for *F. chrysotus*. The intercepts are significantly different from each other ($P<.001$).

These anatomical relationships and statistical comparisons are reviewed in Tables 18 and 19.

Figure 23. Dry weight of gills of F. *similis* as a function of body weight. Slope = 0.9874 ± 0.0545. Intercept = 0.8940 ± 0.0201. Correlation coefficient = 0.89.

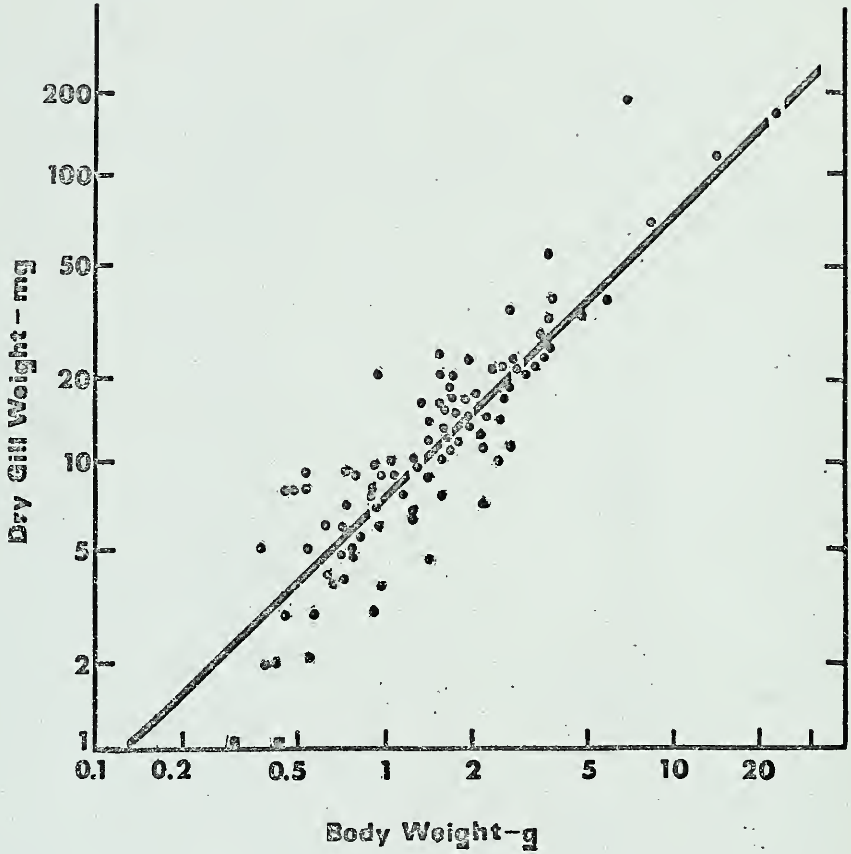
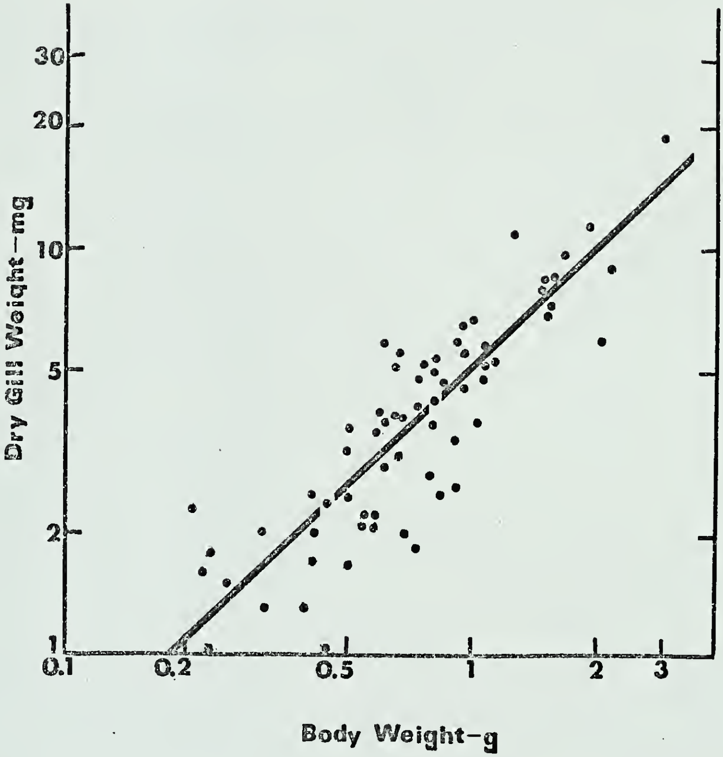

Dry Gill Weight – mg
200
100
50
20
10
5
2
1
0.1
0.2
0.5
1
2
5
10
20
Body Weight–g

Figure 24. Dry weight of gills of *F. chrysotus* as a function of body weight. Slope = 0.9671 ± 0.1022. Intercept = 0.7021 ± 0.0284. Correlation coefficient = 0.65.


Dry Gill Weight−mg
30
20
10
5
2
1
0.1
0.2
0.5
1
2
3
Body Weight−g

DISCUSSION

The capacity for precise osmoregulation is required of fish living in waters of rapidly fluctuating salinity if conforming to the external concentration means trespass across the internal limits of tolerance. In waters of stable salinity, there is little advantage in being able to osmoregulate over a wide range of salinity, especially if such osmoregulatory ability requires additional energy to maintain the involved mechanisms. *F. similis*, which naturally lives in waters of widely varying salinity, can regulate blood plasma concentration precisely. Large populations of *F. chrysotus* inhabit fresh water only. This species possesses a less precise pattern of regulation. In the fresh-water environment, individuals may be effective stenohaline hyperregulators, but they are not necessarily the best regulators over a wide range of salinities.

The concentration of the blood plasma of *F. similis*, living in sea water, is ca. 390 mosm/liter. This is comparable to other values for salt-water fishes (Garrey, 1905; Dakin, 1908; Duval, 1925; Black, 1951; Vinogradov, 1953; Black, 1957; Hickman, 1959). The concentration of the blood plasma of *F. chrysotus*, living in fresh water, is 246 mosm/liter. This is comparable to, but slightly lower than, values for other fresh-water teleosts (Duval, 1925; Krogh, 1939; Black, 1951; Robertson, 1954; Black, 1957). This slight depression may result from a reduced energetic expenditure in fresh water.

The pattern of regulation found for *F. similis* has been reported for other teleosts (Duval, 1925; Keys, 1933; Hickman, 1959; Gordon, 1964; Valentine and Miller, 1969). The pattern of a gradual increase in blood plasma concentration up to some critical salinity, followed by a sudden decrease, was shown for *F. chrysotus*. Lotan (1966) has previously described a similar pattern for *Tilapia aurea* and has concluded that the sudden decrease in blood plasma concentration may be the result of decreased permeability. The depressed oxygen consumption of intact *F. chrysotus* in full sea water may be the result of decreased permeability. Stanley and Fleming (1966) have found this pattern of regulation in *Fundulus kansae* when fish are rapidly transferred from fresh water to water of higher salinity. However, when fish are completely acclimated to salinity, *F. kansae* regulates in a fashion similar to *F. similis*.

F. chrysotus is ecologically a stenohaline fresh-water species, but it possesses a pattern of regulation similar to *T. aurea* and *F. kansae*, both of which are euryhaline species. *F. chrysotus* has the physiological capacity to survive indefinitely in waters of high salinity and its energy expenditure is similar to that of *F. similis*. However, only occasional fish are found under such conditions normally. Some other factor of an ecological or physiological nature, such as competition for food or salinity tolerance of the eggs, must be important in limiting the range which *F. chrysotus* normally inhabits.

A possible error in the blood plasma concentration studies arises from the fact that most samples from *F. similis* were larger than those from *F. chrysotus*. This was unavoidable because of the difference between the size ranges of the two species.

In Tables 6 and 7, salinity-dependent changes in the oxygen consumption of intact animals do not appear to be correlated with changes in osmotic work for the following reasons:

1. Assuming Potts (1954) and Potts and Parry (1964) have correctly estimated the energy necessary for osmotic work, the changes in oxygen consumption of intact animals are too large to be explained by differences in ion transport. Potts (1954) has stated that between 0.3% and 1.2% of the total energy requirement is used for osmotic work. Potts and Parry (1964) have estimated 2% for this value and further have claimed that:

   > ...the increase in metabolism consequent on the increased ion transport would not be detectable against the background of the metabolism of the whole animal. (p. 337)

   F. similis show changes in intact animal oxygen consumption ranging from a 34% decrease for a 0.5 g animal in fresh water to a 64% increase for a 10.0 g animal in sea water. *F. chrysotus* values range from a 31% decrease for a 0.5 g animal in sea water to a 5% increase for a 2.0 g animal in 2/3 sea water.
2. There is no change in the oxygen consumption shown by a 1.0 g *F. chrysotus* in 2/3 sea water even though $|B - M|$ has increased to three times what it was in 1/3 sea water.
3. There are large decreases in oxygen consumption shown by *F. similis* in fresh water and *F. chrysotus* in sea water. If changes in intact animal oxygen consumption are associated with changes in osmotic work, then as

$|B - M|$ increases, oxygen consumption should also increase unless permeability is varying.

Significant changes in the oxygen consumption of intact animals as a function of salinity may result from changes in activity. Both species show the lowest rates of oxygen consumption when placed in water of the salinity which they are least likely to encounter in nature. F. chrysotus in full sea water and F. similis in fresh water have depressed oxygen consumptions relative to values in 1/3 sea water. I have observed that fish under these conditions lie quietly as if under too much stress to move. Spoor (1946) has shown that variations in activity affect the oxygen consumption of fish. Therefore, intact animals under the greatest osmotic stress should consume oxygen at low rates because they are inactive. Because of the magnitudes of the changes in oxygen consumption of intact fish and the fact that they consume the smallest amounts of oxygen when under the greatest osmotic stress, oxygen consumption of intact animals appears to be a function of something other than osmotic work, probably of activity.

The magnitudes of the changes in gill oxygen consumption are more reasonable as estimates of osmotic work. At 20° C, F. similis gills show variations in oxygen consumption ranging from a 46% decrease (4.0% of intact animal oxygen consumption) by a 10.0 g fish in fresh water to an 89% increase (5.9% of intact animal oxygen consumption) by a 0.5 g fish in sea water. Oxygen consumption of F. chrysotus gills at 20° C ranges from a 15.7% decrease (1.1% of intact animal oxygen consumption) by a 0.5 g fish in fresh water to a 54.1% increase (3.1% of intact animal oxygen consumption) by a 2.0 g fish in sea water. Although these changes in oxygen consumption are of the proper size to account for changes in osmotic work, their direction is not always parallel to the changes in $|B - M|$. Some decreases in gill oxygen consumption occur as $|B - M|$ increases. At least two explanations are possible.

1. Potts and Parry (1964) have stated:

   > ...it is possible that transport systems can adjust to different demands either by 'changing gear' at each transport site, or by having a variety of sites of different capacity. (p. 39)

   Thus an identical amount of oxygen consumption can result in various amounts of salts being transported depending on the demands placed on the system.

2. Smith (1967) has suggested that permeability of membranes may vary as a function of salinity. If gill permeability is decreased sufficiently, then it is possible for a decreased energy expenditure to be associated with an increase in $|B - M|$.

Both factors may be responsible for the fact that changes in gill oxygen consumption do not consistently parallel the changes in $|B - M|$. Since this study was not carried out on an ion flux level, the first factor cannot be considered. But gill permeability can be calculated according to Potts (1954), as outlined in the materials and methods section. Because the possibility of a transport pump with a changing efficiency is not considered in this paper, these permeabilities are better termed apparent permeabilities.

A necessary assumption in calculating permeabilities in this manner is that salt gained by the gills equals salt lost by the gills. For a fish in a hypoosmotic medium, this means we must assume that salt lost in the urine equals salt gained through the intestinal tract. Since these two values are small both quantitatively and energetically relative to flux through the gills (Grafflin, 1938; Potts, 1954; Black, 1957), this is a reasonable assumption. In a hyperosmotic medium, the urine of fishes is nearly isosmotic to the blood (Baldwin, 1948). The kidney neither dilutes nor concentrates the blood. Swallowing as an important osmoregulatory mechanism in salt water was first suggested by Smith (1930). Recently, Potts and Evans (1966, 1967) and Motais and Maetz (1964) have questioned the relative importance of drinking sea water. Potts _et al_. (1967) have estimated that 13.3% of sodium influx in _Tilapia mossambica_ is through the gut when the fish is living in 100% sea water. Although drinking is essential in sea water, it is of much less importance quantitatively than gill activity. Because the intestinal tract does play some role in hypoosmotic regulation, work estimated for fish in salt water may be a little low and therefore apparent permeability may also be low.

For both species, the apparent permeability is greatest in water that is nearest to being isosmotic with the blood plasma (Tables 16 and 17). Apparent permeability decreases as $|B - M|$ increases. If we assume that gas, ion, and water permeabilities are related to each other, this suggests that gill permeability is a compromise between gas exchange and osmoregulation. A highly permeable gill is desirable for gas exchange and a relatively impermeable gill is a requirement for precise osmoregulation. When the concentration gradient between the blood plasma and the external medium is low, the fish gill is highly permeable, allowing enhanced gas exchange. However, if the concentration gradient is great, the fish gill becomes more impermeable, decreasing the energy necessary for osmoregulation.

In both species, apparent permeability decreases with increasing body size at all salinities. This result seems reasonable for *F. chrysotus* because its surface area per unit weight increases as body weight increases. *F. similis* shows a decrease in both apparent permeability and weight specific surface area as body weight increases. This enables large members of this species to regulate at reduced energetic costs.

Potts (1954) has given values for KA (surface area X permeability), osmotic work (cal/g-hr) and its percentage of the total energy expenditure. These values are given in Table 20. The KA factor varies from $3.312X10^{-4}$ to $1.42X10^{-2}$ moles/molar concentration difference-hr. In this study, KA varies from $3.85X10^{-4}$ to $3.23X10^{-1}$ moles/molar concentration difference-hr for *F. similis* (Table 21) and from $1.20X10^{-4}$ to $9.62X10^{-3}$ moles/molar concentration difference-hr for *F. chrysotus* (Table 22). Potts has given values for osmotic work varying from $2.41X10^{-4}$ to $1.26X10^{-3}$ cal/g-hr. *F. similis* uses $9.69X10^{-3}$ to $1.21X10^{-1}$ cal/g-hr and *F. chrysotus* uses from $1.90X10^{-2}$ to $6.32X10^{-2}$ cal/g-hr. These values are larger for two reasons:

1. Potts discusses values for 60 g animals. Values given for this study are for *F. similis* weighing from 0.5 g to 10.0 g and for *F. chrysotus* weighing from 0.5 g to 2.0 g. That these values decrease with increasing body size may be seen in Tables 21 and 22.
2. Potts states that his values are thermodynamically minimal for performing the necessary osmotic work.

Osmotic work varies as a percentage of the total work expenditure from

TABLE 20

ENERGY EXPENDITURE FOR OSMOREGULATION (POTTS, 1954)

Species	KA* (M/ M-hr)	Osmotic work (cal/g-hr)	% total energy expenditure
Potambius	2.307×10^{-4}	6.11×10^{-4}	0.3%
Eriocher	3.312×10^{-4}	1.26×10^{-3}	0.5%
Anodonta	1.420×10^{-2}	2.41×10^{-4}	1.2%

* Permeability times surface area in moles per molar concentration difference per hour.

TABLE 21

ENERGY EXPENDITURE OF *F. similis* FOR OSMOREGULATION

Salinity	KA* (M/M-hr)	Gill Activity (cal/g-hr)	% total energy expenditure
		(0.50 g fish)	
f	6.17×10^{-4}	7.62×10^{-2}	14.9%
1/3	5.76×10^{-2}	7.36×10^{-2}	8.3%
2/3	7.61×10^{-4}	9.60×10^{-2}	12.3%
3/3	3.85×10^{-4}	1.21×10^{-1}	12.6%
		(2.00 g fish)	
f	9.45×10^{-4}	2.93×10^{-2}	8.8%
1/3	1.28×10^{-1}	3.52×10^{-2}	9.3%
2/3	1.35×10^{-3}	4.23×10^{-2}	10.2%
3/3	4.66×10^{-4}	4.22×10^{-2}	7.8%
		(10.0 g fish)	
f	1.55×10^{-3}	9.69×10^{-3}	4.8%
1/3	3.23×10^{-1}	1.78×10^{-2}	10.7%
2/3	2.58×10^{-3}	1.63×10^{-2}	8.3%
3/3	6.79×10^{-4}	1.25×10^{-2}	4.6%

*Permeability times surface area in moles per molar concentration difference per hour.

TABLE 22

ENERGY EXPENDITURE OF *F. chrysotus* FOR OSMOREGULATION

Salinity	KA* (M/ M-hr)	Gill Activity (cal/g-hr)	% total energy expenditure
	(0.50 g fish)		
f	4.44×10^{-4}	5.30×10^{-2}	5.88%
1/3	8.01×10^{-3}	6.30×10^{-2}	6.69%
2/3	1.18×10^{-3}	5.60×10^{-2}	6.27%
3/3	1.20×10^{-4}	6.32×10^{-2}	9.69%
	(1.00 g fish)		
f	5.63×10^{-4}	3.35×10^{-2}	5.80%
1/3	8.78×10^{-3}	3.45×10^{-2}	5.80%
2/3	1.67×10^{-3}	3.96×10^{-2}	6.65%
3/3	1.62×10^{-4}	4.30×10^{-2}	9.32%
	(2.00 g fish)		
f	7.02×10^{-4}	2.10×10^{-2}	5.69%
1/3	9.62×10^{-3}	1.90×10^{-2}	5.03%
2/3	2.36×10^{-3}	2.80×10^{-2}	7.07%
3/3	2.21×10^{-4}	2.92×10^{-2}	8.88%

*Permeability times surface area in moles per molar concentration difference per hour.

0.3% to 1.2% according to Potts. In the present study, oxygen consumption of excised gills varies from 4.6% to 14.9% of the total oxygen consumption for F. similis and from 5.0% to 9.7% of the total oxygen consumption for F. chrysotus. These values are also higher than those of Potts due to differences in sizes of the animals and the efficiencies of the systems.

Data in Table 21 and 22 suggest that F. similis pays a higher price for its precise pattern of osmoregulation than F. chrysotus pays for its sloppy pattern of regulation. Comparing fish of equal sizes, the differences in energetic costs can be estimated. The gills of 0.5 g and 2.0 g F. similis utilize more energy than gills of 0.5 g and 2.0 g F. chrysotus at all salinities. The difference ranges from 1.06×10^{-2} to 5.89×10^{-2} cal/g-hr for a 0.5 g fish and from 0.83×10^{-2} to 1.62×10^{-2} cal/g-hr for a 2.0 g fish. As body size increases, the discrepancy between the price for precise regulation and sloppy regulation decreases.

Changes in the oxygen consumption of intact animals do not consistently parallel changes in the oxygen consumption of excised gills because they are directly dependent upon different variables. Oxygen consumption of intact animals depends primarily upon the degree of muscular activity (Spoor, 1946). That activity is a function of salinity has been suggested in a previous paragraph by the fact that animals under the greatest osmotic stress are inactive and consume small amounts of oxygen. It is assumed that a major factor determining the oxygen consumption of excised gills is the amount of osmotic work being performed which in turn is a function of salinity. Although oxygen consumption of excised gills is a function of osmotic work, the relationship is not a simple one as the efficiency of transport pumps and permeability may also vary as a function of salinity. Intact animals and excised gills consume oxygen at rates which indirectly depend on salinity. However, the factors upon which those rates directly depend are distinct.

The effect of temperature on the standard oxygen consumption of fishes has been shown to be independent of body size (Job, 1955; Beamish and Mookherjii, 1964). Hickman (1959) has made the only study of the effect of body size on the salinity-oxygen consumption relationship in fishes. His data indicate that body size is an important factor, but he only considers two salinities. The present study suggests that the effect of salinity on the oxygen consumption of both intact animals and excised gills is a function of body size. Since intact animal oxygen consumption depends on muscular activity and oxygen consumption of excised gills on osmotic work, the effect of salinity on muscular activity and osmotic work must also be a function of body size. Whenever possible, body size should be considered in osmoregulatory studies.

Conclusions

In any homeostatic system, the energy expenditure may be varied in order to control the level of the system in the face of external fluctuations. However, the constant of proportionality may also be varied. This may lead to energy changes which are more than proportional or less than proportional to changes in external levels. It is even possible to find decreases in the energy expenditure as the difference between the level of a parameter in an animal and the environment increases if the constant of proportionality is decreased sufficiently.

In this study, osmoregulatory ability is shown to be a result of varying the energy expenditure and also varying the apparent permeability. Energy expenditure varies both as a function of $|B - M|$ and as a function of body size. Apparent permeability, likewise, is a function of both $|B - M|$ and body size. Good osmoregulation results when proper adjustments in these two factors are made as the concentration of the medium varies.

Summary

1. F. similis which inhabits salt marsh tidal creeks is a precise osmoregulator over the range of salinity from fresh to full sea water. The fresh-water F. chrysotus has a less precise pattern of osmoregulation over the same range. This pattern is similar to those of the euryhaline fishes, Tilapia aurea (Lotan, 1966) and Fundulus kansae (Stanley and Fleming, 1966). F. chrysotus has the physiological capacity to osmoregulate in a manner similar to some euryhaline species, but it is stenohaline in an ecological sense.
2. Oxygen consumption of intact animals varies significantly as a function of salinity. However, these changes cannot be correlated with the energetic costs for osmotic work because they are up to 30 times the minimum amount necessary to perform osmotic work as estimated by Potts (1954). This indicates an efficiency of ca. 3% which is much lower than the efficiencies (20% to 80%) of other transport systems (Potts and Parry, 1964).

It is concluded that salinity may cause an activity response which results in changes in oxygen consumption of intact animals. Oxygen consumption of excised gills shows significant variations in _F_. _similis_ and nonsignificant variations in _F_. _chrysotus_ as a function of salinity. The magnitude of these changes correlates well with the amount of osmotic work which must be performed. The direction of these changes does not consistently parallel the direction of the changes in $|B - M|$. This may be the result of a transport system which changes its level of efficiency or of a varying permeability.

3. Both species show decreases in apparent gill permeability as $|B - M|$ increases. Apparent permeability decreases as a function of increasing body size at all salinities.
4. Changes in the oxygen consumption of intact animals do not consistently parallel changes in the oxygen consumption of excised gills. It is suggested that the oxygen consumption of intact animals is a function of the effect of salinity on activity, while the oxygen consumption of isolated gills reflects the amount of osmotic work. Body size significantly affects the salinity-oxygen consumption relationship of both intact animals and excised gills.

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BIOGRAPHICAL SKETCH

Dale Frederick Burnside was born May 15, 1943, at Cincinnati, Ohio. He graduated from Walnut Hills High School in June, 1961. Mr. Burnside entered the University of Cincinnati in September, 1961 and received a Bachelor of Arts degree with high honors in June, 1965. In September, 1965, he entered the Zoology Department at the University of Florida and served one year as a graduate teaching assistant. During the summer of 1966, Mr. Burnside attended the National Science Foundation organism-sediment seminar in Bermuda. Upon his return to the University of Florida, Mr. Burnside continued studies toward the degree of Doctor of Philosophy with the support of a National Defense Education Act Title IV Fellowship. He participated in the National Defense Education Act traveling scholar program during the summer of 1967, when he attended a course in the physiology of fishes at the University of South Florida. In the spring of 1969, he presented a paper to the Florida Academy of Sciences.

Mr. Burnside is married to the former Laura Virginia Sjodahl. He is a member of Phi Beta Kappa and Phi Sigma.

This dissertation was prepared under the direction of the chairman and the co-chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1969

E. Ruffin Jones
Dean, College of Arts and Sciences

Dean, Graduate School

Supervisory Committee:

Chairman

Co-chairman

Daniel A. Belkin

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